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<p>(21) International Application Number: PCT/US99/25954</p> <p>(22) International Filing Date: 4 November 1999 (04.11.99)</p> <p>(30) Priority Data: 60/106,976 4 November 1998 (04.11.98) US</p> <p>(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application US 60/106,976 (CIP) Filed on 4 November 1998 (04.11.98)</p> <p>(71) Applicant (for all designated States except US): THE GOVERNMENT OF THE UNITED STATES OF AMERICA, as represented by THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES [US/US]; National Institutes of Health, Office of Technology Transfer, Suite 325, 6011 Executive Boulevard, Rockville, MD 20852-3804 (US).</p> <p>(72) Inventors; and (75) Inventors/Applicants (for US only): LENARDO, Michael, J. [US/US]; 9117 Falls Chapel Way, Potomac, MD 20854 (US). WANG, Jin [CN/US]; Apartment 203, 13208 Twinbrook Parkway, Rockville, MD 20851 (US). JIANG, Di</p>	<p>[CN/US]; 9310 Rhode Island Avenue, College Park, MD 20714 (US).</p> <p>(74) Agents: SPRATT, Gwendolyn, D. et al.; Needle & Rosenberg, P.C., Suite 1200, The Candler Building, 127 Peachtree Street N.E., Atlanta, GA 30303-1811 (US).</p> <p>(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</p> <p>Published <i>Without international search report and to be republished upon receipt of that report.</i></p>																																																																									
<p>(54) Title: A NOVEL TUMOR NECROSIS FACTOR FAMILY MEMBER, DRL, AND RELATED COMPOSITIONS AND METHODS</p>																																																																										
<p>(57) Abstract</p> <p>The present invention provides an isolated nucleic acids encoding DRL proteins, DRL-1 and DRL-2, as set forth in the Sequence Listing as SEQ ID NO:1 and SEQ ID NO:3, respectively. The invention also provides a unique fragment of SEQ ID NO:1 that encodes amino acid residues 65 to 250 of DRL-1 as set forth in SEQ ID NO:2. The invention further provides purified polypeptides encoded by the nucleic acids encoding the DRL proteins as set forth in SEQ ID NO:1 and SEQ ID NO:3, purified polypeptide having the sequences set forth in the sequence listing as SEQ ID NO:2, SEQ ID NO:4 and SEQ ID NO:6 and of DRL polypeptides which can be used to stimulate an immune response or induce apoptosis. In another aspect the invention provides purified antibodies which bind to the DRL protein or fragments thereof. These antibodies can be used to inhibit a T cell response, to inhibit apoptosis or to suppress an autoimmune response.</p>																																																																										
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**A NOVEL TUMOR NECROSIS FACTOR FAMILY
MEMBER, DRL, AND RELATED COMPOSITIONS AND METHODS**

FIELD OF THE INVENTION

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Specifically, the invention relates to isolated nucleic acids encoding DRL (death receptor ligand) proteins, to unique fragments of the nucleic acid encoding DRL proteins, and to the polypeptides encoded by those nucleic acids. The invention further relates to antibodies to those polypeptides. The invention also relates to methods of
10 utilizing the nucleic acids, polypeptides and antibodies of the invention for modulating immune responses.

BACKGROUND ART

15 Lymphocyte apoptosis is critical for the maintenance of peripheral tolerance. Molecules such as CD95 (Fas/APO-1) and other molecules related to tumor necrosis factor (TNF) and TNF receptor (TNFR) have been shown to play a critical role in lymphocyte apoptosis and peripheral tolerance. Mice deficient in Fas or Fas ligand develop enlarged secondary lymphoid organs, accumulation of CD3+CD4-CD8- T
20 cells, increase in total B cell number and production of autoantibodies. TNF and TNFR type I may also contribute to autoimmunity. This suggests that TNF and TNFR family members play an important role in lymphocyte apoptosis and maintenance of peripheral tolerance. With the exception of lymphotoxin β which lacks a transmembrane domain, all TNF family members are expressed on the cell surface as type II membrane
25 proteins. The extracellular domains of the TNF family members are most conserved among all the members and interact with their receptors. TNFR members are type I membrane proteins with characteristic cysteine-rich repeats in the extracellular domain. The intracellular regions of TNFR members are divergent and mediate different biological signals into the cells.

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The members of the TNF family are expanding. Existing members include TNF- α , lymphotoxin- α (LT- α), lymphotoxin- β (LT- β), TRANCE/RANKL, LIGHT, TRAIL/APO-2 ligand, TWEAK, and ligands for CD27, CD30, CD40, OX40, 4-1BB, and CD95/Fas/APO-1. Interactions of TNF family members with their receptors

5 induce diverse biological functions besides apoptosis, including cell proliferation and differentiation. Mice deficient in LT- α or LT- β display significant defects in lymph nodes and spleen, suggesting that LT- α and LT- β are required for the development of peripheral lymphoid organs. The interaction of CD40 on B cells and CD40 ligand on T cells has been shown to be important for B cell maturation and activation, and for T cell

10 proper functions. Interactions of TRANCE/RANKL on T cells with its receptors on dendritic cells stimulates both the antigen presentation ability of dendritic cells and T cell growth. In contrast, Fas ligand and TNF- α have been shown to be critical for apoptosis of mature lymphocytes. Mice deficient in Fas ligand or its receptor, Fas, display dramatic lymphocyte accumulation in the peripheral lymphoid tissues and

15 autoimmunity. TNFR1 deficiency enhances the severity of symptoms resulting from Fas mutation. Thus, Fas ligand and TNF appear to be two of the major inducers of apoptosis in the TNF family. TRANCE/RANKL, TRAIL/APO-2 ligand, and TWEAK are capable of inducing apoptosis in certain cell lines, and may induce apoptosis in mature lymphocytes under specific conditions. Thus ligands in the TNF family may

20 have diverse stimulatory and inhibitory functions in lymphocytes as well as other cell-types alone or in combination with other lymphokines, cytokines, chemokines or other stimulatory agents. Various cytokines, chemokines or lymphokine enhancers and inhibitors that are generally known (*Paul, W.E. (ed.) Fundamental Immunology* (3rd ed.) New York Raven, 1993) may be employed to enhance or inhibit the activities of

25 DRL-1 or DRL-2.

Novel genes with extensive homology to the TNF family have been identified and cloned. One gene is named DRL-1 for *death receptor ligand-1*. It may also be referred to herein as TNF-gamma or TNF-like ligand (TLL, pronounced "tall"). The

30 other gene is named DRL-2.

SUMMARY OF THE INVENTION

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The present invention provides isolated nucleic acids encoding the human DRL-1 protein and the human DRL-2 protein as set forth in the Sequence Listing as SEQ ID NO: 1 and SEQ ID NO: 3, respectively. The invention also provides a unique fragment of the isolated nucleic acid that encodes amino acid residues 65 to 250 of DRL-1 as set forth in SEQ ID NO:2. The present invention provides an isolated nucleic acid encoding the mouse DRL-1 protein as set forth in the Sequence Listing as SEQ ID NO:5.

The invention further provides purified polypeptides encoded by the nucleic acids encoding the DRL protein as forth in SEQ ID NO:1, SEQ ID NO:3 and SEQ ID NO:5, purified polypeptides having the sequence set forth in the sequence listing as SEQ ID NO:2, SEQ ID NO:4 and SEQ ID NO:6 and of DRL polypeptides which can be used to stimulate an immune response or induce apoptosis.

In another aspect the invention provides purified antibodies which bind to the DRL protein or fragments thereof. These antibodies can be used to inhibit a T cell response, to inhibit apoptosis or to suppress an autoimmune response. These antibodies can also be used as agonists to stimulate the membrane-bound form of the ligand.

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BRIEF DESCRIPTION OF THE FIGURES

Figure 1A shows that amino acid sequence of DRL-1. The amino acid sequence of DRL-1 suggests that it is a type 2 membrane protein. The putative transmembrane domain is underlined and corresponds to amino acids 26-50 of SEQ ID NO:2. The asterisk indicates the position of the predicted N-glycosylation site, corresponding to

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residue 124 of SEQ ID NO:2. The arrow marks the N-terminus of the extracellular domain, beginning with residue 65 of SEQ ID NO:2, used to generate a soluble version of DRL-1.

5 Figure 1B shows a sequence alignment of extracellular receptor-binding regions of DRL-1 and several other members of the TNF family. The extracellular domain of DRL-1 with extensive homology to other TNF family members is shown. Alignment was performed using the Megalign software (DNASTAR) and the shading represents identical residues.

10

Figure 2 shows a northern blot analysis of DRL-1. (A) and (B) Blots of RNA from a variety of human tissues or PBLs were purchased and detected with DRL-1 or actin probes followed by autoradiography.

15 Figure 3 shows the genomic localization of DRL-1. (A) DNA prepared from chromosomes 1 to 23, total human DNA (+) or H₂O (-) was used as template for PCR of DRL-1. The primers are described in the Examples in the section *Identification, cloning and sequencing analysis of DRL-1*. PCR products were analyzed by agarose gel electrophoresis. (B) A diagram showing six different chromosome 17 deletion
20 DNA panels. Lines indicate the chromosome 17 sequence that is present in the samples. (C) PCR products of different chromosome 17 deletion panels shown in (B) or H₂O (-) using the primer as in (A).

25 Figure 4 shows the purification of soluble DRL-1. DRL-1-Fc purified by anti-Flag column was dialyzed against PBS and 20 μ l of the eluent was used for SDS-PAGE followed by coomassie blue staining. (A) Molecular weight marker. (B) DRL-1-Fc.

Figure 5 shows the staining of cell lines with DRL-1-Fc. Cells were incubated with DRL-1-Fc and then FE-conjugated goat F(ab')₂ anti-human IgG-Fc. The cells were then analyzed by flow cytometry.

5 Figure 6 shows the predicted protein sequence of DRL-2. The underlined sequence indicates a region likely to be essential for protein function. This fragment corresponds to residues 184-209 of SEQ ID NO:4. The peptide sequence of this region can be used to raise polyclonal and monoclonal antibodies against DRL-2.

10 Figure 7 shows the expression of DRL-2 in spleen and peripheral blood lymphocytes (PBL). A probe specific for DRL-2 sequence was used to probe human mRNA membranes.

15 Figure 8 shows the expression of the cDNAs for DRL-1 and DRL-2. DRL-1 and DRL-2 encode discrete proteins of different sizes. DRL-2 encodes two isoforms.

Figure 9 shows the analysis of the protein regions of DRL-1.

20 Figure 10 shows the analysis of the protein regions of DRL-2.

Figure 11 illustrates the relationship between DRL-1 and DRL-2 and the relationship of DRL-1 and DRL-2 to other members of the TNF gene family.

25 Figure 12 shows the chromosomal mapping of DRL-2 to chromosome 13.

DESCRIPTION OF THE INVENTION

The present invention may be understood more readily by reference to the following detailed description of the preferred embodiments of the invention and the
30 Example included therein.

Before the present compounds and methods are disclosed and described, it is to be understood that this invention is not limited to specific proteins or specific methods. It is also to be understood that the terminology used herein is for the purpose of
5 describing particular embodiments only and is not intended to be limiting.

It must be noted that, as used in the specification and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise.

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In one aspect, the invention provides an isolated nucleic acid encoding DRL-1, for example, as set forth in the Sequence Listing as SEQ ID NO:1. This is an example of a cDNA encoding the DRL-1 protein. Another example of a cDNA encoding a DRL-1 protein is set forth in the Sequence Listing as SEQ ID NO:5. SEQ ID NO: 5 is
15 an example of a mouse cDNA of DRL-1. The present invention also provides the gene encoding DRL-1, an example of which is contained within the disclosed sequence corresponding to EMBL/GenBank/DDBJ accession no. AF046888. The term "gene" as used herein means a unit of heredity that occupies a specific locus on a chromosome as well as any sequences associated with the expression of that nucleic acid. For example,
20 the gene includes any introns normally present within the coding region as well as regions preceding and following the coding region. Examples of these non-coding regions include, but are not limited to transcription termination regions, promoter regions, enhancer regions and modulation regions. Since the genomic location of the DRL-1 gene is provided herein, the present invention includes any examples of the
25 DRL-1 gene that occur at that locus.

In another aspect the present invention provides an isolated nucleic acid encoding DRL-2, for example, as set forth in the Sequence Listing as SEQ ID NO:3. This is an example of a cDNA encoding the DRL-2 protein. The present invention also provides the gene encoding DRL-2. Since the genomic location of the DRL-2 gene is provided herein, the present invention includes any examples of the DRL-2 gene that occur at that locus. Similar to DRL-1, DRL-2 is another member of the TNF family, however, the cDNA for DRL-2 provided herein, does not encode an N-terminal hydrophobic stretch that would normally anchor a protein to the membrane as seen in DRL-1 and other TNF family members. Thus, DRL-2 would be directly secreted into the extracellular medium.

As described herein, unless otherwise stated or necessitated by context, "DRL", "DRL protein", "DRL polypeptide", "DRL cDNA" and "DRL gene" can refer to DRL-1 or DRL-2 or both.

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As used herein, the term "isolated" refers to a nucleic acid separated or significantly free from at least some of the other components of the naturally occurring organism, for example, the cell structural components commonly found associated with nucleic acids in a cellular environment and/or other nucleic acids. The isolation of the native nucleic acids can be accomplished, for example, by techniques such as cell lysis followed by phenol plus chloroform extraction, followed by ethanol precipitation of the nucleic acids. The nucleic acids of this invention can be isolated from cells according to any of many methods well known in the art.

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One skilled in the art will appreciate that the cDNA or cDNA fragments (probes, primers etc.) of a DRL protein provide information with which the genomic nucleic acids corresponding to this cDNA, or genomic variants of this gene can be isolated. For example, primers for amplifying the gene that encodes the DRL-1 protein can be designed using the sequence information disclosed in SEQ ID NO: 1. Similarly, primers can be designed using the sequence information disclosed in SEQ ID NO:3 for amplifying the gene that encodes the DRL-2 protein. Alternatively, those same disclosed nucleic acids can be used to design probes for detecting a nucleic acid containing all or part of the genomic nucleic acid in a genomic library.

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As used herein, the terms "genomic variant" and "allelic variant" mean a similar gene in another organism of the same species. For example, a nucleic acid from one member of a species can encode for a particular DRL protein while another member of the same species has a different nucleic acid which encodes for that same DRL protein. A similar gene in another species is defined herein as a "homolog" of a DRL protein. For example, a DRL protein from one species can be different than a DRL protein from another species, yet both may have the same function as the exemplified DRL protein.

As used herein, the term "nucleic acid" refers to single-or multiple stranded molecules which may be DNA or RNA, or any combination thereof, including modifications to those nucleic acids. The nucleic acid may represent a coding strand or its complement, or any combination thereof. Nucleic acids may be identical in sequence to the sequences which are naturally occurring for any of the novel genes discussed herein, or they may include alternative codons which encode the same amino acid as that which is found in the naturally occurring sequence. These nucleic acids can

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also be modified from their typical structure. Such modifications include, but are not limited to, methylated nucleic acids, the substitution of a non-bridging oxygen on the phosphate residue with either a sulfur (yielding phosphorothioate deoxynucleotides), selenium (yielding phosphorselenoate deoxynucleotides), or methyl groups (yielding methylphosphonate deoxynucleotides).

The genes and nucleic acids provided for by the present invention may be obtained in any number of ways. For example, a DNA molecule encoding a DRL protein can be isolated from the organism in which it is normally found. For example, a genomic DNA or cDNA library can be constructed and screened for the presence of the gene or nucleic acid of interest. Methods of constructing and screening such libraries are well known in the art and kits for performing the construction and screening steps are commercially available (for example, Stratagene Cloning Systems, La Jolla, CA). Once isolated, the gene or nucleic acid can be directly cloned into an appropriate vector, or if necessary, be modified to facilitate the subsequent cloning steps. Such modification steps are routine, an example of which is the addition of oligonucleotide linkers which contain restriction sites to the termini of the nucleic acid. General methods are set forth in *Sambrook et al.*, "Molecular Cloning, a Laboratory Manual," Cold Spring Harbor Laboratory Press (1989).

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Once the gene or nucleic acid sequence of the desired DRL protein is obtained, the sequence encoding specific amino acids can be modified or changed at any particular amino acid position by techniques well known in the art. For example, PCR primers can be designed which span the amino acid position or positions and which can substitute any amino acid for another amino acid. Then a nucleic acid can be amplified

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and inserted into the wild-type DRL protein coding sequence in order to obtain any of a number of possible combinations of amino acids at any position of the DRL protein. Alternatively, one skilled in the art can introduce specific mutations at any point in a particular nucleic acid sequence through techniques for point mutagenesis. General methods are set forth in *Smith, M.* "In vitro mutagenesis" *Ann. Rev. Gen.*, 19:423-462 (1985) and *Zoller, M.J.* "New molecular biology methods for protein engineering" *Curr. Opin. Struct. Biol.*, 1:605-610 (1991). Techniques such as these can also be used to modify the genes or nucleic acids in regions other than the coding regions, such as the promoter regions for the DRL protein. Likewise, these techniques can be used to alter the coding sequence without altering the amino acid sequence that is encoded.

Another example of a method of obtaining a DNA molecule encoding a specific DRL protein is to synthesize a recombinant DNA molecule which encodes the DRL protein. For example, oligonucleotide synthesis procedures are routine in the art and oligonucleotides coding for a particular protein region are readily obtainable through automated DNA synthesis. A nucleic acid for one strand of a double-stranded molecule can be synthesized and hybridized to its complementary strand. One can design these oligonucleotides such that the resulting double-stranded molecule has either internal restriction sites or appropriate 5' or 3' overhangs at the termini for cloning into an appropriate vector. Double-stranded molecules coding for relatively large proteins can readily be synthesized by first constructing several different double-stranded molecules that code for particular regions of the protein, followed by ligating these DNA molecules together. For example, *Cunningham, et al.*, "Receptor and Antibody Epitopes in Human Growth Hormone Identified by Homolog-Scanning Mutagenesis," *Science*, 243:1330-1336 (1989), have constructed a synthetic gene encoding the human

growth hormone gene by first constructing overlapping and complementary synthetic oligonucleotides and ligating these fragments together. See also, *Ferretti, et al.*, Proc. Nat. Acad. Sci. 82:599-603 (1986), wherein synthesis of a 1057 base pair synthetic bovine rhodopsin gene from synthetic oligonucleotides is disclosed. By constructing a

5 DRL protein in this manner, one skilled in the art can readily obtain any particular DRL protein with desired amino acids at any particular position or positions within the DRL protein. See also, U.S. Patent No. 5,503,995 which describes an enzyme template reaction method of making synthetic genes. Techniques such as this are routine in the art and are well documented. These nucleic acids or fragments of a nucleic acid

10 encoding a DRL protein can then be expressed *in vivo* or *in vitro* as discussed below.

The invention also provides for the isolated nucleic acids of SEQ ID NO:1, SEQ ID NO: 3, and SEQ ID NO:5 in a vector suitable for expressing the nucleic acid. Once a nucleic acid encoding a particular DRL protein of interest, or a region of that

15 nucleic acid, is constructed, modified, or isolated, that nucleic acid can then be cloned into an appropriate vector, which can direct the *in vivo* or *in vitro* synthesis of that wild-type and/or modified DRL protein. The vector is contemplated to have the necessary functional elements that direct and regulate transcription of the inserted gene, or nucleic acid. These functional elements include, but are not limited to, a promoter, regions

20 upstream or downstream of the promoter, such as enhancers that may regulate the transcriptional activity of the promoter, an origin of replication, appropriate restriction sites to facilitate cloning of inserts adjacent to the promoter, antibiotic resistance genes or other markers which can serve to select for cells containing the vector or the vector containing the insert, RNA splice junctions, a transcription termination region, or any

other region which may serve to facilitate the expression of the inserted gene or hybrid gene. (See generally, *Sambrook et al.*).

There are numerous *E. coli* (*Escherichia coli*) expression vectors known to one
5 of ordinary skill in the art which are useful for the expression of the nucleic acid insert. Other microbial hosts suitable for use include bacilli, such as *Bacillus subtilis*, and other enterobacteriaceae, such as *Salmonella*, *Serratia*, and various *Pseudomonas* species. In these prokaryotic hosts one can also make expression vectors, which will typically contain expression control sequences compatible with the host cell (e.g., an
10 origin of replication). In addition, any number of a variety of well-known promoters will be present, such as the lactose promoter system, a tryptophan (Trp) promoter system, a beta-lactamase promoter system, or a promoter system from phage lambda. The promoters will typically control expression, optionally with an operator sequence, and have ribosome binding site sequences for example, for initiating and completing
15 transcription and translation. If necessary, an amino terminal methionine can be provided by insertion of a Met codon 5' and in-frame with the downstream nucleic acid insert. Also, the carboxy-terminal extension of the nucleic acid insert can be removed using standard oligonucleotide mutagenesis procedures.

20 Additionally, yeast expression can be used. There are several advantages to yeast expression systems. First, evidence exists that proteins produced in a yeast secretion systems exhibit correct disulfide pairing. Second, post-translational glycosylation is efficiently carried out by yeast secretory systems. The *Saccharomyces cerevisiae* pre-pro-alpha-factor leader region (encoded by the *MF"-1* gene) is routinely
25 used to direct protein secretion from yeast. (*Brake, et al.*, A%-Factor-Directed

Synthesis and Secretion of Mature Foreign Proteins in *Saccharomyces cerevisiae*.@
Proc. Nat. Acad. Sci., 81:4642-4646 (1984)). The leader region of pre-pro-alpha-factor
contains a signal peptide and a pro-segment which includes a recognition sequence for
a yeast protease encoded by the *KEX2* gene: this enzyme cleaves the precursor protein
5 on the carboxyl side of a Lys-Arg dipeptide cleavage signal sequence. The nucleic acid
coding sequence can be fused in-frame to the pre-pro-alpha-factor leader region. This
construct is then put under the control of a strong transcription promoter, such as the
alcohol dehydrogenase I promoter or a glycolytic promoter. The nucleic acid coding
sequence is followed by a translation termination codon which is followed by
10 transcription termination signals. Alternatively, the nucleic acid coding sequences can
be fused to a second protein coding sequence, such as Sj26 or β -galactosidase, used to
facilitate purification of the fusion protein by affinity chromatography. The insertion of
protease cleavage sites to separate the components of the fusion protein is applicable to
constructs used for expression in yeast. Efficient post translational glycosylation and
15 expression of recombinant proteins can also be achieved in Baculovirus systems.

Mammalian cells permit the expression of proteins in an environment that
favors important post-translational modifications such as folding and cysteine pairing,
addition of complex carbohydrate structures, and secretion of active protein. Vectors
20 useful for the expression of active proteins in mammalian cells are characterized by
insertion of the protein coding sequence between a strong viral promoter and a
polyadenylation signal. The vectors can contain genes conferring hygromycin
resistance, gentamicin resistance, or other genes or phenotypes suitable for use as
selectable markers, or methotrexate resistance for gene amplification. The chimeric
25 protein coding sequence can be introduced into a Chinese hamster ovary (CHO) cell

line using a methotrexate resistance-encoding vector, or other cell lines using suitable selection markers. Presence of the vector DNA in transformed cells can be confirmed by Southern blot analysis. Production of RNA corresponding to the insert coding sequence can be confirmed by Northern blot analysis. A number of other suitable host

5 cell lines capable of secreting intact human proteins have been developed in the art, and include the CHO cell lines, HeLa cells, myeloma cell lines, Jurkat cells, etc.

Expression vectors for these cells can include expression control sequences, such as an origin of replication, a promoter, an enhancer, and necessary information processing sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and

10 transcriptional terminator sequences. Preferred expression control sequences are promoters derived from immunoglobulin genes, SV40, Adenovirus, Bovine Papilloma Virus, etc. The vectors containing the nucleic acid segments of interest can be transferred into the host cell by well-known methods, which vary depending on the type of cellular host. For example, calcium chloride transformation is commonly utilized

15 for prokaryotic cells, whereas calcium phosphate, DEAE dextran, or lipofectin mediated transfection or electroporation may be used for other cellular hosts.

Alternative vectors for the expression of genes or nucleic acids in mammalian cells, those similar to those developed for the expression of human gamma-interferon,

20 tissue plasminogen activator, clotting Factor VIII, hepatitis B virus surface antigen, protease NexinI, and eosinophil major basic protein, can be employed. Further, the vector can include CMV promoter sequences and a polyadenylation signal available for expression of inserted nucleic acids in mammalian cells (such as COS-7).

Insect cells also permit the expression of mammalian proteins. Recombinant proteins produced in insect cells with baculovirus vectors undergo post-translational modifications similar to that of wild-type proteins. Briefly, baculovirus vectors useful for the expression of active proteins in insect cells are characterized by insertion of the protein coding sequence downstream of the *Autographica californica* nuclear polyhedrosis virus (AcNPV) promoter for the gene encoding polyhedrin, the major occlusion protein. Cultured insect cells such as *Spodoptera frugiperda* cell lines are transfected with a mixture of viral and plasmid DNAs and the viral progeny are plated. Deletion or insertional inactivation of the polyhedrin gene results in the production of occlusion negative viruses which form plaques that are distinctively different from those of wild-type occlusion positive viruses. These distinctive plaque morphologies allow visual screening for recombinant viruses in which the AcNPV gene has been replaced with a hybrid gene of choice. High quantity expression and production of a DRL protein can also be achieved by transgenic animal technology by which animals can be made to produce DRL in serum, milk, etc in large amounts.

The invention also provides for the vectors containing the contemplated nucleic acids in a host suitable for expressing the nucleic acids. The vectors containing the nucleic acid segments of interest can be transferred into host cells by well-known methods, which vary depending on the type of cellular host. For example, calcium chloride transformation, transduction, and electroporation are commonly utilized for prokaryotic cells, whereas calcium phosphate, DEAE dextran, or lipofection mediated transfection or electroporation may be used for other cellular hosts.

Alternatively, the genes or nucleic acids of the present invention can be operatively linked to one or more of the functional elements that direct and regulate transcription of the inserted gene as discussed above and the gene or nucleic acid can be expressed. For example, a gene or nucleic acid can be operatively linked to a bacterial
5 or phage promoter and used to direct the transcription of the gene or nucleic acid *in vitro*. A further example includes using a gene or nucleic acid provided herein in a coupled transcription-translation system where the gene directs transcription and the RNA thereby produced is used as a template for translation to produce a polypeptide. One skilled in the art will appreciate that the products of these reactions can be used in
10 many applications such as using labeled RNAs as probes and using polypeptides to generate antibodies or in a procedure where the polypeptides are being administered to a cell or a subject.

Expression of the gene or nucleic acid, in combination with a vector, can be by
15 either *in vivo* or *in vitro*. *In vivo* synthesis comprises transforming prokaryotic or eukaryotic cells that can serve as host cells for the vector. Alternatively, expression of the gene or nucleic acid can occur in an *in vitro* expression system. For example, *in vitro* transcription systems are commercially available which are routinely used to synthesize relatively large amounts of mRNA. In such *in vitro* transcription systems,
20 the nucleic acid encoding the DRL protein would be cloned into an expression vector adjacent to a transcription promoter. For example, the Bluescript II cloning and expression vectors contain multiple cloning sites which are flanked by strong prokaryotic transcription promoters. (Stratagene Cloning Systems, La Jolla, CA). Kits are available which contain all the necessary reagents for *in vitro* synthesis of an RNA
25 from a DNA template such as the Bluescript vectors. (Stratagene Cloning Systems, La

Jolla, CA). RNA produced *in vitro* by a system such as this can then be translated *in vitro* to produce the desired DRL protein. (Stratagene Cloning Systems, La Jolla, CA).

Additionally, contemplated by the present invention are nucleic acids, from any
5 desired species, preferably mammalian and more preferably human, having 99.9%,
99.7%, 99.5%, 99.3%, 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%,
88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%,
73%, 72%, 71%, 70%, 60%, or 50% overall homology or homology in the region being
compared to the same region of a gene comprising a nucleic acid set forth in SEQ ID
10 NO:1, SEQ ID NO: 3 or SEQ ID NO:5 or to a gene comprising a nucleic acid encoding
the polypeptide set forth in SEQ ID NO:2, SEQ ID NO:4 or SEQ ID NO:6 or to an
isolated nucleic acid consisting of the nucleic acid set forth in SEQ ID NO:1, SEQ ID
NO: 3 or SEQ ID NO:5 or to an isolated nucleic acid that encodes the polypeptide set
forth in SEQ ID NO:2, SEQ ID NO:4 or SEQ ID NO:6 of the sequence listing, or to
15 allelic variants or homologs thereof. The assessment of homology is preferably based
on a base-by-base comparison of the regions being compared. These genes and nucleic
acids can be synthesized or obtained by the same methods used to isolate homologs,
with stringency of hybridization and washing, if desired, reduced accordingly as
homology desired is decreased, and further, depending upon the G-C or A-T richness of
20 any area wherein variability is searched for. Allelic variants of any of the present genes
and nucleic acids or of their homologs can readily be isolated and sequenced by
screening additional libraries following the examples given herein and procedures well
known in the art.

The gene or nucleic acid encoding any selected protein of the present invention can be any nucleic acid that functionally encodes that protein. A nucleic acid encoding a selected protein can readily be determined based upon the amino acid sequence of the selected protein, and, clearly, many nucleic acids will encode any selected protein.

5

The present invention additionally provides a nucleic acid that selectively hybridizes under stringent conditions with a gene comprising a nucleic acid set forth in SEQ ID NO:1, SEQ ID NO:3 or SEQ ID NO:5. This hybridization can be specific. The degree of complementarity between the hybridizing nucleic acid and the sequence to which it hybridizes should be at least enough to exclude hybridization with a nucleic acid encoding an unrelated protein. Thus, a nucleic acid that selectively hybridizes with a nucleic acid of a present protein coding sequence will not selectively hybridize under stringent conditions with a nucleic acid for a different, unrelated protein, and vice versa. The temperature and salt conditions are readily determined empirically in preliminary experiments in which samples of reference DNA immobilized on filters are hybridized to a labeled nucleic acid of interest and then washed under conditions of different stringencies.

Hybridization temperatures are typically higher for DNA-RNA and RNA-RNA hybridizations. The washing temperatures can be used as described above to achieve selective stringency, as is known in the art. (See, for example, *Sambrook et al.*, "Molecular Cloning: A Laboratory Manual" 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York (1989) and *Kunkel et al.* *Methods Enzymol.* 1987:154:367 (1987)). Nucleic acid fragments that selectively hybridize to any given nucleic acid can be used, *e.g.*, as primers and or probes for further hybridization or for

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amplification methods (*e.g.*, polymerase chain reaction (PCR), ligase chain reaction (LCR)).

The present invention also contemplates polynucleotide probes for detecting a
5 DRL gene, wherein the polynucleotide probe hybridizes to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1, SEQ ID NO:3 or SEQ ID NO:5. As used herein, the term "polynucleotide probe" refers to a nucleic acid fragment that selectively hybridizes under stringent conditions with a nucleic acid comprising a nucleic acid set forth in a sequence listed herein. This hybridization must be specific.
10 The degree of complementarity between the hybridizing nucleic acid and the sequence to which it hybridizes should be at least enough to exclude hybridization with a nucleic acid encoding an unrelated protein.

Thus, allelic variants can be identified and isolated by nucleic acid hybridization
15 techniques. Probes selective to the nucleic acid set forth in the Sequence Listing as SEQ ID NO:1, SEQ ID NO:3 and SEQ ID NO:5 can be synthesized and used to probe the nucleic acid from various cells, tissues, libraries etc. High sequence complementarity and stringent hybridization conditions can be selected such that the probe selectively hybridizes to allelic variants of the sequence set forth in the Sequence
20 Listing as SEQ ID NO:1, SEQ ID NO:3 and SEQ ID NO:5. For example, the selectively hybridizing nucleic acids of the invention can have at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% and 99% complementarity with the segment of the sequence to which it hybridizes. The nucleic
25 acids can be at least 12, 50, 100, 150, 200, 300, 500, 750, or 1000 nucleotides in length.

Thus, the nucleic acid can be a coding sequence for a DRL protein or fragments thereof that can be used as a probe or primer for detecting the presence of these genes. If used as primers, the invention provides compositions including at least two nucleic acids which hybridize with different regions so as to amplify a desired region. Forward
5 primer, 5'TATCGAATTCACAACCTTCTTCCCTTCTGACCA (SEQ ID NO:7) and reversed primer, 5'TATCGAATTCAATGAAAAGGGAAAAGTGAGGAACG (SEQ ID NO:8) are provided as examples of amplification primers. Depending on the length of the probe or primer, target region can range between 70% complementary bases and full complementarity and still hybridize under stringent conditions. For example, for
10 the purpose of detecting the presence of an allelic variant of the sequence set forth SEQ ID NO:1, SEQ ID NO:3 or SEQ ID NO:5, the degree of complementarity between the hybridizing nucleic acid (probe or primer) and the sequence to which it hybridizes is at least enough to distinguish hybridization with a nucleic acid from other species. The invention provides examples of nucleic acids unique to SEQ ID NO:1, SEQ ID NO:3
15 and SEQ ID NO:5 so that the degree of complementarity required to distinguish selectively hybridizing from nonselectively hybridizing nucleic acids under stringent conditions can be clearly determined for each nucleic acid.

"Stringent conditions" refers to the washing conditions used in a hybridization
20 protocol. In general, the washing conditions should be a combination of temperature and salt concentration chosen so that the denaturation temperature is approximately 5-20°C below the calculated T_m of the nucleic acid hybrid under study. The temperature and salt conditions are readily determined empirically in preliminary experiments in which samples of reference DNA immobilized on filters are hybridized
25 to the probe or protein coding nucleic acid of interest and then washed under conditions

of different stringencies. The T_m of such an oligonucleotide can be estimated by allowing 2°C for each A or T nucleotide, and 4°C for each G or C. For example, an 18 nucleotide probe of 50% G+C would, therefore, have an approximate T_m of 54°C.

5 The present invention also contemplates any unique fragment of these genes or of the nucleic acids set forth in SEQ ID NO:1, SEQ ID NO:3 and SEQ ID NO:5. To be unique, the fragment must be of sufficient size to distinguish it from other known sequences, most readily determined by comparing any nucleic acid fragment to the nucleotide sequences of nucleic acids in computer databases, such as GenBank. Such
10 comparative searches are standard in the art. Typically, a unique fragment useful as a primer or probe will be at least about 20 to about 25 nucleotides in length, depending upon the specific nucleotide content of the sequence. Additionally, fragments can be, for example, at least about 30, 40, 50, 75, 100, 200, 210, 211, 212, 213, 214, 215, 216, 220, 230, 240, 245, 246, 247, 248, 249, 250, 300, 350, 400, 500, 600, 700, 800, 900,
15 1000 nucleotides in length or any number in between. All of the genes, nucleic acids, and fragments of the genes and nucleic acids disclosed and contemplated herein can be single or double stranded, depending upon the purpose for which it is intended.

The invention also contemplates compounds comprising the genes, nucleic
20 acids, and fragments of the DRL genes and nucleic acids as disclosed and contemplated herein. For example, a compound comprising a nucleic acid can be a derivative of a typical nucleic acid such as nucleic acids which are modified to contain a terminal or internal reporter molecule and/or those nucleic acids containing non-typical bases or sugars. These reporter molecules include, but are not limited to, isotopic and non-
25 isotopic reporters. Examples include, a FLAG tag or a human IgG Fc. Therefore any

molecule which may aid in detection, amplification, replication, expression, purification, uptake, etc. may be added to the nucleic acid construct.

The invention also provides for an isolated double-stranded nucleic acid
5 amplified using primer pair 5'-TATCGAATTCACAACCTTCTTCCCTTCTGCACCA
(SEQ ID NO:7) and 5' TATCGAATTCAATGAAAAGGGAAAAGTGAGGAACG
(SEQ ID NO:8) consisting of 1) single-stranded DNA which has a molecular size of
approximately 1.3 Kb and is derived from humans, and 2) a DNA complementary to
the single-stranded DNA.

10

Further provided are single-stranded RNAs corresponding to the 1.3 Kb single-stranded DNA and the single-stranded 1.3 Kb complementary DNA, respectively.

DNA sizes can be measured by several means including, running the nucleic
15 acid on a gel such as an agarose or polyacrylamide gel, or by other molecular size
estimates techniques, such as molecular weight filtration. Briefly, the nucleic acid
sample is applied to the gel and electrophoresed simultaneously with nucleic acid
markers of known molecular size. Upon completion of gel electrophoresis, the size of
the nucleic acid sample is estimated by comparing its mobility with the relative
20 mobilities of the nucleic acid markers.

In another aspect, the invention provides a polypeptides encoded by the nucleic
acid set forth in SEQ ID NO:1, SEQ ID NO:3 and SEQ ID NO:5 and the polypeptides
set forth in the Sequence Listing as SEQ ID NO:2, SEQ ID NO:4 and SEQ ID NO:6.
25 The invention also provides fragments of unmodified and modified DRL proteins. The

polypeptide fragments of the present invention can be recombinant proteins obtained by cloning nucleic acids encoding the polypeptide in an expression system capable of producing the polypeptide fragments thereof. For example, one skilled in the art can determine the active regions of a DRL protein which can interact with another protein
5 and cause a biological effect associated with the DRL protein. In one example, amino acids found to not contribute to either the activity, binding specificity, or other biological effect associated with the DRL protein can be deleted and /or substituted without a loss in the respective activity. The fragments, whether attached to other sequences or not, can also include insertions, deletions, substitutions, or other selected
10 modifications of particular regions or specific amino acid residues, provided the activity of the peptide is not significantly altered or impaired.

Further contemplated are polypeptides encoded by fragments of the DRL nucleic acids provided herein. An example of an active fragment of DRL-1 is the
15 extracellular domain of DRL shown in Fig.1. It is noted that the DRL-1 protein fragment shown in SEQ ID NO:2 is a specific example of a fragment of DRL-1 corresponding to nucleotides 193 to 750, and that other examples of DRL and DRL fragments having slightly different sequences may be found in nature using routine protocols or generated by design. The DRL protein fragments encoded by nucleotides
20 1 to 193 of SEQ ID NO: 1 are also contemplated by this invention as are the protein fragments encoded by nucleotides 1 to 699 or SEQ ID NO:5 and the protein fragments encoded by nucleotides 1 to 654 or SEQ ID NO:3. Additionally, DRL-1 fragments comprising or consisting of amino acids 26-50 of SEQ ID NO:2 or a fragment beginning with amino acid 65 of SEQ ID NO:2 are provided. DRL-2 fragments are
25 also provided, including a peptide comprising residues 184-209 of SEQ ID NO:4.

Additional fragments of DRL-1 and DRL-2 can be identified based on there
correspondence to functional regions of other previously known members of the TNF
family. Identification of these regions in DRL is made routine by the provision of
exemplary sequences of DRL-1 and DRL-2 herein and their comparison to published
5 sequences of other members of the family (see Figure 1B, for example).

By "active fragment" is meant a subpart of a whole DRL protein that exhibits an
activity of DRL. For example, the fragment can stimulate and enhance immune
responses such as those against viral, bacterial, parasitic and toxic agent. The fragment
10 can also enhance the intensity of immune responses at certain stages to induce or
augment antigen-stimulated immune responses or lymphocyte death. The latter may be
more important for limiting the intensity or duration of an immune response and/or
preventing the development of potential autoimmune reactions. The active fragment
can also enhance or stimulate anti-tumor immunity. The DRL fragment can be used in
15 combination with other immune response modifiers to increase or decrease their effect
on immune responses. The behavior of any cell bearing an appropriate receptor can be
modified by the DRL fragment by contacting said receptor and stimulating a cellular
response in a manner that promotes function and preserves or restores health.

20 These DRL polypeptides can also be obtained in any of a number of procedures
well known in the art. One method of producing a polypeptide is to link two peptides
or polypeptides together by protein chemistry techniques. For example, peptides or
polypeptides can be chemically synthesized using currently available laboratory
equipment using either Fmoc (9-fluorenylmethyloxycarbonyl) or Boc (*tert*
25 -butyloxycarbonyl) chemistry. (Applied Biosystems, Inc., Foster City, CA). One

skilled in the art can readily appreciate that a peptide or polypeptide corresponding to a particular protein can be synthesized by standard chemical reactions. For example, a peptide or polypeptide can be synthesized and not cleaved from its synthesis resin whereas the other fragment of a hybrid peptide can be synthesized and subsequently
5 cleaved from the resin, thereby exposing a terminal group which is functionally blocked on the other fragment. By peptide condensation reactions, these two fragments can be covalently joined via a peptide bond at their carboxyl and amino termini, respectively, to form a larger polypeptide. (*Grant, ASynthetic Peptides: A User Guide,*@ W.H. Freeman and Co., N.Y. (1992) and *Bodansky and Trost, Ed.,*
10 *APrinciples of Peptide Synthesis,*@ Springer-Verlag Inc., N.Y. (1993)). Alternatively, the peptide or polypeptide can be independently synthesized *in vivo* as described above. Once isolated, these independent peptides or polypeptides may be linked to form a larger protein via similar peptide condensation reactions.

15 For example, enzymatic ligation of cloned or synthetic peptide segments can allow relatively short peptide fragments to be joined to produce larger peptide fragments, polypeptides or whole protein domains (*Abrahmsen et al. Biochemistry, 30:4151 (1991)*). Alternatively, native chemical ligation of synthetic peptides can be utilized to synthetically construct large peptides or polypeptides from shorter peptide
20 fragments. This method consists of a two step chemical reaction (*Dawson et al. A Synthesis of Proteins by Native Chemical Ligation,*@ *Science, 266:776-779 (1994)*). The first step is the chemoselective reaction of an unprotected synthetic peptide-%-thioester with another unprotected peptide segment containing an amino-terminal Cys residue to give a thioester-linked intermediate as the initial
25 covalent product. Without a change in the reaction conditions, this intermediate

undergoes spontaneous, rapid intramolecular reaction to form a native peptide bond at the ligation site. Application of this native chemical ligation method to the total synthesis of a protein molecule is illustrated by the preparation of human interleukin 8 (IL-8) (*Clark-Lewis et al.* FEBS Lett., 307:97 (1987), *Clark-Lewis et al.*, J.Biol.Chem., 5 269:16075 (1994), *Clark-Lewis et al.* Biochemistry, 30:3128 (1991), and *Rajaratnam et al.* Biochemistry, 29:1689 (1994)).

Alternatively, unprotected peptide segments can be chemically linked where the bond formed between the peptide segments as a result of the chemical ligation is an 10 unnatural (non-peptide) bond (*Schnolzer et al.* Science, 256:221 (1992)). This technique has been used to synthesize analogs of protein domains as well as large amounts of relatively pure proteins with full biological activity (*deLisle Milton et al.* A Techniques in Protein Chemistry IV, @ Academic Press, New York, pp. 257-267 (1992)).

15

Also provided herein are purified antibodies that selectively or specifically bind to the DRL polypeptides provided and contemplated herein, for example, purified antibodies which selectively or specifically bind to a polypeptide encoded by the nucleic acid set forth in any of SEQ ID NO:1, SEQ ID NO:3 or SEQ ID NO:5, and 20 purified antibodies which selectively or specifically bind to the polypeptide set forth in SEQ ID NO:2, SEQ ID NO:4 or SEQ ID NO:6. The antibody (either polyclonal or monoclonal) can be raised to any of the polypeptides provided and contemplated herein, both naturally occurring and recombinant polypeptides, and immunogenic fragments, thereof. For example, an anti-DRL-1 antibody can be raised to the 25 extracellular domain of DRL-1, beginning with residue 65 of SEQ ID NO:2, and used

herein to generate a soluble version of DRL-1. The antibody can be used in techniques or procedures such as diagnostics, treatment, or vaccination. Anti-idiotypic antibodies and affinity matured antibodies are also considered.

- 5 For example, the invention provides monoclonal antibodies designated MAb1, MAb2, MAb3, Mab4 and Mab5 that specifically bind DRL-1.

- Antibodies against DRL-2 are provided. Antibodies can be raise against the whole DRL-2 molecule or against immunogenic fragments of it. For example, a
10 fragment corresponds to residues 184-209 of SEQ ID NO:4 can be used to raise polyclonal and monoclonal antibodies against DRL-2.

- The purified antibodies of this invention include monoclonal antibodies which can be used for diagnostic or analytical purposes. For example, the monoclonal
15 antibody could be utilized in a clinical testing kit to monitor levels of DRL in human tissues or secretions.

- Antibodies can be made by many well-known methods (See, e.g. *Harlow and Lane*, "Antibodies; A Laboratory Manual" Cold Spring Harbor Laboratory, Cold
20 Spring Harbor, New York, (1988)). Briefly, purified antigen can be injected into an animal in an amount and in intervals sufficient to elicit an immune response. Antibodies can either be purified directly, or spleen cells can be obtained from the animal. The cells can then fused with an immortal cell line and screened for antibody secretion. The antibodies can be used to screen nucleic acid clone libraries for cells
25 secreting the antigen. Those positive clones can then be sequenced. (See, for example,

Kelly *et al. Bio/Technology*, 10:163-167 (1992); Bebbington *et al. Bio/Technology*, 10:169-175 (1992)). Humanized and chimeric antibodies are also contemplated in this invention. Heterologous antibodies can be made by well known methods (See, for example, US Patents 5545806, 5569825, 5625126, 5633425, 5661016, 5770429, 5 5789650, and 5814318)

The phrase "specifically binds" with the polypeptide refers to a binding reaction which is determinative of the presence of the protein in a heterogeneous population of proteins and other biologics. Thus, under designated immunoassay conditions, the 10 specified antibodies bound to a particular protein do not bind in a significant amount to other proteins present in the sample. Selective binding to an antibody under such conditions may require an antibody that is selected for its specificity for a particular protein. A variety of immunoassay formats may be used to select antibodies that selectively bind with a particular protein. For example, solid-phase ELISA 15 immunoassays are routinely used to select antibodies selectively immunoreactive with a protein. See *Harlow and Lane "Antibodies, A Laboratory Manual"* Cold Spring Harbor Publications, New York, (1988), for a description of immunoassay formats and conditions that could be used to determine selective binding.

20 This invention also contemplates producing a selected cell line or a non-human transgenic animal model for the analysis of the function of a gene comprising introducing into an embryonic stem cell a vector having a selectable marker which, when the vector is inserted within a gene, the inserted vector can inhibit the expression of the gene, selecting embryonic stem cells expressing the selectable marker, excising 25 the vector from the embryonic stem cells expressing the selectable marker such that

host DNA from the gene is linked to the excised vector, sequencing the host DNA in the excised vector, comparing the sequence of the host DNA to known gene sequences to determine which host DNA is from a gene for which a model for the analysis of the function the gene is desired, selecting the embryonic stem cell containing the inhibited
5 gene for which a model for the analysis of gene function is desired, and forming a cell line or a non-human transgenic animal from the selected embryonic stem cell.

It is also contemplated in this invention that transgenic animals can be produced which either overproduce the polypeptides of this invention or fail to produce the
10 polypeptides of this invention in a functional form. For example, a transgenic animal which overproduces a DRL of this invention can be produced according to methods well known in the art whereby nucleic acid encoding DRL is introduced into embryonic stem cells, at which stage it is incorporated into the germline of the animal, resulting in the production of DRL in the transgenic animal in increased amounts relative to a
15 normal animal of the same species. One skilled in the art can determine if overproduction or underproduction of DRL results in altered immune responses.

A transgenic animal in which the expression of DRL, for example, is tissue specific is also contemplated for this invention. For example, transgenic animals that
20 express or overexpress these genes at specific sites such as the liver can be produced by introducing a nucleic acid into the embryonic stem cells of the animal, wherein the nucleic acid is under the control of a specific promoter which allows expression of the nucleic acid in specific types of cells (e.g., a hepatic promoter which allows expression only in hepatic cells. One skilled in the art can determine if a tissue-specific alteration
25 in DRL expression results in altered immune responses.

Alternatively, the transgenic animal of this invention can be a “knock out” animal (see, e.g., Willnow et al., 1996), which can be an animal that, for example, normally produces DRL but has been altered to prevent the expression of the animal’s nucleic acid which encodes DRL, thereby resulting in an animal which does not
5 produce DRL in a functional form. Such an animal may lack the ability to express all of the nucleic acids encoding DRL or the transgenic animal may lack the ability to express some (one or more than one) but not all of the nucleic acids encoding the DRL.

For example, the transgenic “knock out” animal of this invention can have the
10 expression of a gene or genes knocked out in specific tissues. This approach obviates viability problems that can be encountered if the expression of a widely expressed gene is completely abolished in all tissues. One skilled in the art could determine whether or not the “knock out” has altered the animal’s immune response by comparing the “knock out” animal with control animals that have not had DRL knocked out in a
15 specific tissue.

Also contemplated in this invention is a method of stimulating an immune response in a subject by administering DRL and a pharmaceutically acceptable carrier. Alternatively, an active fragment of DRL, or a DRL encoding nucleic acid in an
20 expressible construct, can be administered. For any of the methods described herein for using DRL or DRL fragments, nucleic acids encoding DRL or its fragments can be administered. Both DRL-1 and DRL-2 can also be used to prepare highly active trimeric versions of the molecule for clinical use using either a modified leucine zipper motif (*Walczak et al.* “Tumoricidal activity of tumor necrosis factor-related apoptosis-
25 inducing ligand in vivo” *Nat. Med.* 5:157-63 (1999)) or a trimeric coiled-coil motif

from gp41 (*Shu et al.* "Trimerization specificity in HIV-1gp41: analysis with a GCN4 leucine zipper model" *Biochemistry* 27:5378-85 (1999)).

The subject which can be treated by this method can be any animal. In a preferred embodiment, the animal of the present invention is a human. In addition, non-human animals which can be treated by the methods of this invention can include, but are not limited to, cats, dogs, birds, horses, cows, goats, sheep, guinea pigs, hamsters, gerbils and rabbits.

Optimal dosages used will vary according to the individual being treated and the DRL being used. The amount of DRL will also vary among individuals on the basis of age, size, weight, condition, etc. One skilled in the art will realize that dosages are best optimized by the practicing physician and methods for determining dose amounts and regimens and preparing dosage forms are described, for example, in *Remington's Pharmaceutical Sciences*. For example, suitable doses and dosage regimens can be determined by comparison to agents presently used in the treatment or prevention of autoimmune disorders, allergic disorders, graft rejection and other T cell related disorders. The preferred or optimal dosage is the amount of DRL which results in apoptosis of T cells, in the absence of significant side effects.

20

Typically, the DRL of this invention can be administered orally or parenterally in a dosage range of 0.1 to 100 mg/kg of body weight depending on the clinical response that is to be obtained. Administration of DRL can be stopped completely following a prolonged remission or stabilization of disease signs and symptoms and readministered following a worsening of either the signs or symptoms of the disease, or

25

following a significant change in immune status, as determined by routine follow-up immunological studies well known to a clinician in this field.

The efficacy of administration of a particular dose of DRL in treating a T cell associated disorder as described herein can be determined by evaluating the particular aspects of the medical history, the signs, symptoms and objective laboratory tests that have a documented utility in evaluating pathophysiological activity of the particular T cell associated disorder being treated. These signs, symptoms and objective laboratory tests will vary depending on the particular disorder being treated, as will be well known to any clinician in this field. For example, if, based on a comparison with an appropriate control group and knowledge of the normal progression of the disorder in the general population or the particular individual, 1) a subject's frequency or severity of recurrences is shown to be improved; 2) the progression of the disease or disorder is shown to be stabilized; or 3) the need for use of other immunosuppressive medications is lessened, then a particular treatment can be considered efficacious.

In a particular example, in using the DRLs of the present invention to treat an autoimmune disorder such as multiple sclerosis, clinical parameters and symptoms which can be monitored for efficacy can include the severity and number of attacks; or for continuously progressive disease, the worsening of symptoms and signs; the cumulative development of disability; the number or extent of brain lesions as determined by magnetic resonance imaging; and the use of immunosuppressive medications (65-67).

Once it is established that disease activity is significantly improved or stabilized by a particular DRL treatment, specific signs, symptoms and laboratory tests can be evaluated in accordance with a reduced or discontinued treatment schedule. If a disease activity recurs, based on standard methods of evaluation of the particular signs, symptoms and objective laboratory tests as described herein, DRL treatment can be reinitiated.

Additionally, the efficacy of administration of a particular dose of a peptide ligand in preventing a T cell associated disorder in a subject not known to have a T cell associated disorder, but known to be at risk of developing a T cell associated disorder, can be determined by evaluating standard signs, symptoms and objective laboratory tests, known to one of skill in the art, over time. This time interval may be long (i.e., years/decades). The determination of who would be at risk for the development of a T cell associated disorder would be made based on current knowledge of the known risk factors for a particular disorder familiar to clinicians and researchers in this field, such as a particularly strong family history of a disorder or exposure to or acquisition of factors or conditions which are likely to lead to development of a T cell associated disorder.

By "pharmaceutically acceptable" is meant a material that is not biologically or otherwise undesirable, i.e., the material may be administered to an individual along with the selected compound without causing any undesirable biological effects or interacting in a undesirable manner with any of the other components of the pharmaceutical composition in which it is contained. The carrier may depend on the method of administration and the particular patient. Methods of administration can be

oral, sublingual, mucosal, inhaled, absorbed, or by injection. It is also noted that not all methods of administering the present DRL polypeptides or nucleic acids require a pharmaceutically acceptable carrier.

5 As used herein, "immunosuppressed" describes a condition in which the subject's immune system is unable to mount a protective response. There are several forms of immunosuppression. These include but are not limited to the immune suppression of B cells resulting in the inability of these cells to produce immunoglobulin heavy chains, thereby abrogating antibody production; suppressor T-
10 cell effects on helper and cytotoxic T cells that include inhibition of proliferation, abrogation of lymphokine secretion and clonal anergy. Immunosuppressive effects on cell motility, homing, and cell surface phenotype are also possible.

 In the present invention, the DRL, DRL antibody, or active fragment can be
15 orally or parenterally administered in a carrier pharmaceutically acceptable to human subjects. Suitable carriers for oral or inhaled administration of DRL can include one or more of the carriers pharmaceutically acceptable to human subjects. Suitable carriers for oral administration of DRL include one or more substances which may also act as a flavoring agents, lubricants, suspending agents, or as protectants. Suitable solid
20 carriers include calcium phosphate, calcium carbonate, magnesium stearate, sugars, starch, gelatin, cellulose, carboxypolymethylene, or cyclodextrans. Suitable liquid carriers may be water, pyrogen free saline, pharmaceutically accepted oils, or a mixture of any of these. The liquid can also contain other suitable pharmaceutical addition such as buffers, preservatives, flavoring agents, viscosity or osmo-regulators, stabilizers or
25 suspending agents. Examples of suitable liquid carriers include water with or without

various additives, including carboxypolymethylene as a pH-regulated gel. The DRL may be contained in enteric coated capsules that release the polypeptide into the intestine to avoid gastric breakdown. For parenteral administration of the antagonist, a sterile solution or suspension is prepared in saline that may contain additives, such as
5 ethyl oleate or isopropyl myristate, and can be injected for example, into subcutaneous or intramuscular tissues, as well as intravenously.

Similarly, using gene therapy methods, a nucleic acid encoding DRL or functional fragment can be administered. The nucleic acid encoding the peptide of this
10 invention can be placed into a vector and delivered to the cells of a subject either *in vivo* or *ex vivo* by standard methods.

For *in vivo* administration, the cells can be in a subject and the nucleic acid can be administered in a pharmaceutically acceptable carrier. The subject can be any
15 animal in which it is desirable to selectively express a nucleic acid in a cell. In a preferred embodiment, the animal of the present invention is a human. In addition, non-human animals which can be treated by the method of this invention can include, but are not limited to, cats, dogs, birds, horses, cows, goats, sheep, guinea pigs, hamsters, gerbils and rabbits, as well as any other animal in which selective expression
20 of a nucleic acid in a cell can be carried out according to the methods described herein.

DRL-1 and/or DRL-2 could be used to modulate immune cell activity *in vitro* in the context of gene therapy, bone marrow transplantation, and in cultivating tumor-infiltrating lymphocytes for the treatment of cancer. For example, T cells can be
25 collected from the patient and treated *ex vivo* with DRL-1 and/or DRL-2, washed to

remove the DRL, then readministered to the patient. This approach offers the advantage of reducing any side-effects of administration of DRL *in vivo*. Another example of an *ex vivo* application of the present invention would be the expansion of tumor infiltrating lymphocytes of cytolytic T cells by administering a DRL antibody.

- 5 This antibody can be used to expand these T cells for a longer time period *in vitro* to obtain large numbers of cells for adoptive transfer into a patient. Adoptive immunotherapy procedures are known in the art. See, for example, Rosenberg S.A., "Adoptive Immunotherapy for Cancer", *Scientific American*, pp.62-69, May, 1990 and U.S. Pat. No. 5,229,115, hereby incorporated by reference.

10

- In the method described above which includes the introduction of exogenous DNA into the cells of a subject (i.e., gene transduction or transfection), the nucleic acids of the present invention can be in the form of naked DNA or the nucleic acids can be in a vector for delivering the nucleic acids to the cells for expression of the nucleic acid inside the cell. The vector can be a commercially available preparation, such as an
- 15 adenovirus vector (Quantum Biotechnologies, Inc. (Laval, Quebec, Canada). Delivery of the nucleic acid or vector to cells can be via a variety of mechanisms. As one example, delivery can be via a liposome, using commercially available liposome preparations such as Lipofectin®, Lipofectamine® (GIBCO-BRL, Inc., Gaithersburg,
- 20 MD), Superfect® (Qiagen, Inc. Hilden, Germany) and Transfectam® (Promega Biotec, Inc., Madison, WI), as well as other liposomes developed according to procedures standard in the art. In addition, the nucleic acid or vector of this invention can be delivered *in vivo* by electroporation, the technology for which is available from Genetronics, Inc. (San Diego, CA) as well as by means of a Sonoporation machine
- 25 (ImaRx Pharmaceutical Corp., Tucson, AZ).

As one example, vector delivery can be via a viral system, such as a retroviral vector system which can package a recombinant retroviral genome. The recombinant retrovirus can then be used to infect and thereby deliver nucleic acid to the infected cells. The exact method of introducing the nucleic acid into mammalian cells is, of course, not limited to the use of retroviral vectors. Other techniques are widely available for this procedure including the use of adenoviral vectors, adeno-associated viral (AAV) vectors, lentiviral vectors, pseudotyped retroviral vectors, and pox virus vectors, such as vaccinia virus vectors. Physical transduction techniques can also be used, such as liposome delivery and receptor-mediated and other endocytosis mechanism. This invention can be used in conjunction with any of these or other commonly used gene transfer methods.

The nucleic acid and the nucleic acid delivery vehicles of this invention, (e.g., viruses; liposomes, plasmids, vectors) can be in a pharmaceutically acceptable carrier for *in vivo* administration to a subject. By "pharmaceutically acceptable" is meant a material that is not biologically or otherwise undesirable, i.e., the material may be administered to a subject, along with the nucleic acid or vehicle, without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the pharmaceutical composition in which it is contained. The carrier would naturally be selected to minimize any degradation of the active ingredient and to minimize any adverse side effects in the subject, as would be well known to one of skill in the art.

The nucleic acid or vehicle may be administered orally, parenterally (e.g., intravenously), by intramuscular injection, by intraperitoneal injection, transdermally,

extracorporeally, topically or the like. The exact amount of the nucleic acid or vector required will vary from subject to subject, depending on the species, age, weight and general condition of the subject, the severity or mechanism of any disorder being treated, the particular nucleic acid or vehicle used, its mode of administration and the
5 like.

The invention also contemplates a method of suppressing an autoimmune response in a subject by administering an antibody to DRL and a pharmaceutically acceptable carrier. Alternatively, an antibody to a functional region of DRL could be
10 administered.

Since DRL-1 enhances T cell responses, then the blockade of DRL-1 in autoimmune or allergic conditions may lessen disease severity to the extent that the diseases depend on T cell reactions. Also, DRL may be overactive in autoimmune
15 disease and the development of specific antibodies will allow the detection and perhaps inhibition of DRL in these contexts. Similar approaches could be used to suppress allograft rejection to enhance the success of tissue transplantation.

As used herein, "suppressing" describes the inhibition of the autoimmune
20 response which can range from reduction to complete amelioration of the autoimmune response. Measurement of a suppressed immune response involves determining the level of response of peripheral T cells to a specific antigen or in a mixed lymphocyte reaction, important in the context of a disease by assaying the proliferation, cytokine or lymphokine production, or cytotoxic response. It could also involve measuring the
25 levels of specific antibodies, the degree of inflammation in relevant affected body sites,

or decreases in non-specific inflammatory markers that are present in the circulation or in tissues.

Truncated or amino-acid substituted versions of DRL-1 or DRL-2 can also be
5 engineered that can bind the receptor but not cause stimulatory activities. These genetically altered variants may have dominant interfering properties.

As used herein, "autoimmune response" describes a disease state or syndrome whereby a subject's body produces a dysfunctional immune response against the
10 subject's own body components, with adverse effects. This may include production of B cells which produce antibodies with specificity for all antigens, allergens or major histocompatibility (MHC) antigens, or it may include production of T cells bearing receptors that recognize self-components and produce cytokines that cause inflammation. Examples of autoimmune diseases include, but are not limited to,
15 ulcerative colitis, Crohn's disease, multiple sclerosis, rheumatoid arthritis, diabetes mellitus, pernicious anemia, autoimmune gastritis, psoriasis, Bechet's disease, Wegener's granulomatosis, Sarcoidosis, autoimmune thyroiditis, autoimmune oophoritis, bullous pemphigoid, pemphigus, polyendocrinopathies, Still's disease, Lambert-Eaton myasthenia syndrome, myasthenia gravis, Goodpasture's syndrome,
20 autoimmune orchitis, autoimmune uveitis, systemic lupus erythematosus, Sjogren's Syndrome and ankylosing spondylitis.

Alternatively, an antisense molecule to the mRNA of DRL can be administered. Antisense technology is well known in the art and describes a mechanism whereby a
25 nucleic acid comprising a nucleotide sequence which is in a complementary,

“antisense” orientation with respect to a coding or “sense” sequence of an endogenous gene is introduced into a cell, whereby a duplex forms between the antisense sequence and its complementary sense sequence. The formation of this duplex results in inactivation of the endogenous gene. Antisense nucleic acid can be produced for any
5 endogenous gene for which the coding sequence has been or can be determined according to well known methods.

Antisense nucleic acid can inhibit gene expression by forming an RNA/RNA duplex between the antisense RNA and the RNA transcribed from a target gene. The
10 precise mechanism by which this duplex formation decreases the production of the protein encoded by the endogenous gene most likely involves binding of complementary regions of the normal sense mRNA and the antisense RNA strand with duplex formation in a manner that blocks RNA processing and translation. Alternative mechanisms include the formation of a triplex between the antisense RNA and duplex
15 DNA or the formation of a DNA-RNA duplex with subsequent degradation of DNA-RNA hybrids by RNase H. Furthermore, an antisense effect can result from certain DNA-based oligonucleotides via triple-helix formation between the oligomer and double-stranded DNA which results in the repression of gene transcription.

20 As used herein, “T cell responses” can include but are not limited to cell activation, cell proliferation and apoptosis.

Also provided is a method of inhibiting T cell responses by administering an antibody to DRL or DRL fragment and a pharmaceutically acceptable carrier.
25 Alternatively, an antisense molecule to the mRNA of DRL can be administered. Also

provided is a method for stimulating the membrane bound form of DRL by administering an antibody to DRL and a pharmaceutically acceptable carrier.

The invention also provides a method of inhibiting the T cell responses associated with transplantation rejection or graft versus host disease by administering an antibody to DRL or DRL fragment and a pharmaceutically acceptable carrier. Alternatively, an antisense molecule to the mRNA of DRL can be administered.

As used herein, "graft-versus-host" (GvH) disease describes a disease state or syndrome whereby an immune response is initiated by grafted cells and is directed against the subject's body with adverse effects. Examples of GvH disease include, but are not limited to, acute and chronic GvH disease following bone marrow transplant.

As used herein, "transplantation rejection" describes a disease state or syndrome whereby the transplant recipient's body produces an immune response against the engrafted tissue, resulting in rejection. Transplantation rejection can occur, for example, with kidney, heart, lung or liver transplants as well as with any other transplanted tissue.

The invention further provides a method of inducing apoptosis in a population of T cells by administering DRL to the cells. Alternatively, an active fragment of DRL could be administered. Similarly, using gene therapy methods, a nucleic acid encoding DRL or a functional fragment can be administered. Based on the concept that anti-tumor immunity involves lymphocytes, DRL can be used to boost or induce T cell reactions against tumors

Stimulation of T cells from the TCR induces not only cell activation and proliferation, but also apoptosis under certain conditions. Fas ligand and Fas interactions appear to play a major role in the apoptosis process. TNF is also known to be involved. However, we have observed that blocking the actions of Fas ligand and

5 TNF does not completely abolish antigen-induced apoptosis in human PBLs. This raised the possibility that other molecules besides Fas ligand and TNF may also play a role in activation-induced apoptosis in PBLs.

As used herein "apoptosis" refers to programmed cell death that has distinctive

10 morphological and structural features that are different from those of pathological cell death or necrosis. The apoptotic process is characterized by nuclear fragmentation and cytoplasmic budding that lead to the formation of apoptotic bodies. These bodies are phagocytosed and destroyed by nearby macrophages. The fragmentation of the cells does not lead to the release of cellular contents, and the phagocytosis of the apoptotic

15 bodies does not lead to inflammation. As a result, cell death can occur without damage to adjacent cells or tissues.

The invention also provides for a method of inhibiting apoptosis in a population of T cells by administering an antibody to DRL or DRL fragment and a

20 pharmaceutically acceptable carrier. Alternatively, an antisense molecule to the mRNA of DRL can be administered.

The invention also provides a modified DRL conjugated to a toxin that may bind and kill tumor cells. These toxins include, but are not limited to, diphtheria toxin,

ricin, and *Pseudomonas* exotoxin. See, for example, U.S. Pat. No. 4545985 and U.S. Pat. No. 5087616, hereby incorporated by reference.

The present invention also provides a method of screening for a DRL receptor
5 agonist comprising, contacting cells that express the DRL receptor with a putative
agonist, detecting an increase in apoptosis in the cells, whereby an increase in apoptosis
indicates a putative agonist having the potential to induce apoptosis; and determining
that the putative agonist identified in step (b) induces apoptosis by binding with DRL
receptor by competitive binding assays, thereby identifying a DRL receptor agonist.
10

The present invention also provides a method of screening for a DRL receptor
antagonist comprising, contacting cells that express the DRL receptor with a putative
antagonist in the presence of DRL, detecting a decrease in apoptosis in the cells
whereby the decrease in apoptosis indicates a putative antagonist having the potential
15 to inhibit apoptosis; and determining that the putative antagonist identified in step (b)
inhibits apoptosis by binding with DRL receptor by competitive binding assays,
thereby identifying a DRL receptor antagonist.

The present invention is more particularly described in the following examples
20 which are intended as illustrative only since numerous modifications and variations
therein will be apparent to those skilled in the art.

EXAMPLES

Isolation of DRL-1 cDNA

A novel sequence with homology to the TNF family was identified from the
5 National Center for Biotechnology Information dbEST data base using a consensus
amino acid sequence of the TNF family. Further BLAST search of the EST data base
with this EST clone resulted in the identification of several other overlapping clones.
Based on the sequence of the clones and the sequence of the known TNF family
members, a contig of a potential novel TNF-like gene was generated. Full length
10 cDNA of this possible novel gene was therefore cloned from a lymphoma cell line,
CEM. Reverse-transcribed cDNAs prepared from CEM cells was prepared. Primers
designed at both ends of the contig sequence were used for amplification of the cDNA
by PCR. PCR generated a 1.3 kb product which was cloned into pcDNA3 vector.
Sequence analysis of multiple clones shows that the cDNA contains a open reading
15 frame encoding a 250 aa protein (Fig. 1A). The gene was named DRL-1 for death
receptor ligand-1. Sequence alignment analysis indicates that DRL-1 is approximately
25% identical to other TNF family members at the amino acid level (Fig. 1B). It
contains a putative transmembrane domain near the N-terminus from aa residues 26 to
51, suggesting that it is a type II membrane like other members of the TNF family.
20 DRL-1 contains a possible N-glycosylation region at aa 122 to 125, suggesting that
functional form of DRL-1 may be glycosylated.

Tissue distribution of DRL-1

Northern blot analysis suggest that DRL-1 is expressed as a 1.8 kb transcript in
25 heart, placenta, lung, kidney and pancreas (Fig. 2A). In the lymphoid system, DRL-1 is

expressed in the peripheral lymphoid organs, including spleen, lymph nodes and peripheral blood lymphocytes (Fig. 2B). Interestingly, DRL-1 transcript is absent from the thymus and fetal liver (Fig. 2B), indicating that DRL-1 is not expressed by immature T cells as well as hemopoietic progenitors in the fetal liver. However, bone marrow RNA contains abundant amount of DRL-1 (Fig. 2B). What cell types express DRL-1 in the bone marrow is unclear. It is possible that certain hemopoietic progenitor lineages in the bone marrow express DRL-1. Alternatively, this could represent DRL-1 expression by mature lymphocytes that are present in the marrow.

10 *Up-regulation of DRL-1 in T cells by TCR stimulation*

All TNF family members have the potential to regulate cell growth or apoptosis. Many TNF family genes are up-regulated in T cells after activation, suggesting that they are involved in different aspects of T cell regulation during immune responses. To find out if DRL is up-regulated after stimulation from the T cell receptor, RNA derived from T cells with or without TCR crosslinking was analyzed for DRL-1 expression. Fig. 2C shows that after 2.5 h of TCR crosslinking, DRL-1 is significantly up-regulated. Interestingly, DRL-1 mRNA is completely down-regulated after 5 h of stimulation. This suggests that DRL-1 may be important for T cell functions during active immune responses and its regulation is tightly regulated.

20

Chromosomal localization of DRL-1

By PCR method using a pair of specific primers for DRL-1, total human DNA and DNA samples from individual human chromosomes were examined. Fig. 3A. shows that PCR of total human DNA generates a 700 bp product. In all individual chromosome samples, only chromosome 17 DNA template gives rise to a PCR product

25

of the same size as total DNA. None of the other Chromosomal DNA samples results in any PCR band. This suggests that DRL-1, unlike TNF, LT- α and LT- β tightly linked to the MHC locus on chromosome 6, is located on chromosome 17. The sizes of the PCR products from genomic DNA are larger in size than that from DRL-1 cDNA, indicating that there is at least one intron between these two primers. Further PCR analysis using a chromosome 17 deletion panel shows that DRL cannot be amplified from two of the deletion panels, B and E (Fig. 3B and C) but appeared in all the other samples. This indicates that DRL-1 is located on the chromosome 17p11.2-12 region. Based on the approximate genomic location of DRL-1, a BAC genomic clone containing DRL-1 was selected and the presence of DRL-1 in this clone was confirmed by PCR analysis. The BAC clone was then used as a probe for a defined localization of DRL by FISH.

Generation of soluble DRL

C-terminal conserved extracellular regions of TNF family members, such as TNF- α and TRAIL/APO-2 ligand, are sufficient for the biological activities of these proteins. It is therefore possible that the extracellular domain of DRL-1 is biologically active. We therefore fused the DRL-1 extracellular domain from aa 65 to 250 at the N-terminus with human IgG Fc. The construct was expressed in COS cells and the fusion protein was purified from COS cell supernatant with a protein A column. A single band of the 50 KD soluble DRL-1-Fc eluted from the column was observed by SDS-PAGE and coomassie blue staining (Figure 4).

Staining of cell lines by DRL-1-Fc

The DRL-1-Fc protein was then used to stain cell lines. Fig. 5 shows that the DRL-1 fusion protein binds to lymphoma cell lines H9 and Jurkat but not CEM. DRL-1 also stains a adenocarcinoma cell line, COLO 205 and a transformed human embryonic cell line, 293. Interestingly, DRL also binds to human PBLs. This suggests that DRL-1 receptor is widely expressed among many cell types. Identification of cell types bearing the receptor for DRL-1 is important because these cells will be important for analysis of DRL-1 functions. These cell types include T cells, B cells, epithelial cells, malignant cells, and other hematopoietic cells.

10 *Identification, cloning and sequencing analysis of DRL-1*

Search of the EST data base reveals a novel sequence with homology to the TNF family in EST clone AA360555. More search using this EST clone reveals multiple overlapping EST clones. Based on a contig generated from these clones, DRL-1 was amplified by PCR from cDNAs derived from a human lymphoma cell line, CEM. PCR primers for DRL-1 amplification are: forward primer, 5'-TATCGAATTCAACCTTCTCCCTTCTGCACCA (SEQ ID NO: 3); reversed primer, 5'-TATCGAATTCAATGAAAAGGGAAAAGTGAGGAACG (SEQ ID NO:4). The PCR product was digested with EcoR I and ligated to EcoR I-digested pcDNA3 vector. Multiple clones were sequenced by method. Lasergene softwares (DNASTAR, Madison, WI) were used to analyze DRL-1 for its properties and alignment with other TNF family members.

Northern blot analysis

Human peripheral blood lymphocytes were stimulated with PHA and interleukin-2 as described. After culture in IL-2 for three weeks, PBLs added to tissue

culture plates with or without 1 µg/ml 64.1 (anti-CD3) coating. The cells were cultured at 37 °C for 2.5 h and RNA was extracted by the RNazol method (Tel-Test, Friendswood, TX). RNA samples were then used for agarose gel (1.4%) electrophoresis and blotted onto a nylon membrane. The blot was probed with DRL-1 probe and actin probes respectively. RNA blots were also obtained from Clontech and probed for DRL-1 and actin as above.

Genomic localization of DRL-1 by PCR

(1059F+1443 reverse)

- 10 DRL-1 was amplified from genomic DNA using the following primer: 5'-CGAGAAGGCCAAGGAAGGCAGGAGACTCTA-3'(SEQ ID NO:9); reverse primer, 5'-CCTTGCCCGGGGAATTATGACACTCAGAAT-3'(SEQ ID NO:10). DNA from each human chromosome and chromosome 17 deletion panels were used as template. Total human DNA was used as a positive control. The PCR product from genomic DNA is larger than that from DRL-1 cDNA, suggesting that there is at least one intron between these two primers.

Production of soluble DRL-1 from COS cells

- 20 DRL-1 extracellular domain from amino acid residue 65 to 250 was fused at N-terminus with human IgG Fc containing the CH2 and CH3 domain. The construct was cloned into a pCMV1 vector (kodak). The plasmid was then used for transfection of COS cells by DEAE-dextran method (Promega). Seventy two hours after transfection, the supernatant of the COS cells was passed through a 0.45 µM filter and purified with a protein A column. The column was then washed with TBS and eluted with 0.1 M glycine, pH 3.0. The eluent (5 ml) was dialyzed five times against PBS over a 48 h

period and then examined by SDS-PAGE. A single 50 KD band was observed in the eluent by coomassie blue staining. The concentration of the purified DRL-1-Fc was quantitated by the Bio-Rad protein assay method (Bio-Rad, Hercules, CA) using bovine albumin as the standard.

5

Production of Anti-DRL-1 Monoclonal Antibodies

Monoclonal antibodies against DRL-1 designated MAb1, MAb2, MAb3, Mab4 and Mab5 were generated by standard methods. A peptide corresponding to the extracellular domain was used as the immunogen to produce the present antibodies.

10 Briefly, the purified immunogen was injected into an animal in an amount and in intervals sufficient to elicit an immune response. Spleen cells were obtained from the animal. The cells were then fused with an immortal cell line and screened for antibody secretion.

15 Antibodies were selected that bind to DRL-1. For example, solid-phase ELISA immunoassays are routinely used to select antibodies selectively immunoreactive with a protein. See *Harlow and Lane "Antibodies, A Laboratory Manual"* Cold Spring Harbor Publications, New York, (1988), for a description of immunoassay formats and conditions that are commonly used to determine selective binding.

20

Isolation of DRL-2 cDNA

The protocol for the isolation of the DRL-2 cDNA is as described above for DRL-1, however, primers specific for DRL-2 are used. Figure 6 shows the predicted protein sequence of DRL-2. The underlined sequence indicates a region that is likely
25 to be essential for DRL-2 function. In addition to the whole DRL-2 molecule, this

underlined sequence can be used to make peptides for the generation of polyclonal and monoclonal antibodies against DRL-2.

Expression of DRL-2

- 5 Northern blot analysis for the expression of DRL-2 was conducted as described above for DRL-1. A probe specific for DRL-2 sequence was used to probe human mRNA. Figure 7 shows the expression of DRL-2 in spleen and peripheral blood lymphocytes (PBL).

10 *Expression of the cDNAs for DRL-1 and DRL-2*

Figure 8 shows the results of an *in vitro* translation assay. DRL-1 and DRL-2 encode discrete proteins of different sizes. DRL-2 encodes two isoforms.

Genomic localization of DRL-2 by PCR

- 15 Genomic localization experiments for DRL-2 were conducted as described above for DRL-1 with primers specific for DRL-2. The results of these experiments indicate that DRL-2 maps to chromosome 13. See Figure 12.

What is claimed is:

1. An isolated nucleic acid comprising the nucleic acid set forth in the Sequence Listing as SEQ ID NO: 1.
2. The isolated nucleic acid of claim 1 in a vector suitable for expressing the nucleic acid.
3. The vector of claim 2 in a host suitable for expressing the nucleic acid.
4. A purified DRL polypeptide encoded by the nucleic acid of claim 1.
5. An isolated nucleic acid encoding the polypeptide of claim 4.
6. The polypeptide of claim 4, having the sequence set forth in the sequence listing as SEQ ID NO:2.
7. A purified antibody which specifically binds to the polypeptide of claim 4.
8. An isolated nucleic acid comprising the nucleic acid set forth in the Sequence Listing as SEQ ID NO: 3.
9. The isolated nucleic acid of claim 8 in a vector suitable for expressing the nucleic acid.

10. The vector of claim 9 in a host suitable for expressing the nucleic acid.
11. A purified DRL polypeptide encoded by the nucleic acid of claim 8.
12. An isolated nucleic acid encoding the polypeptide of claim 11.
13. The polypeptide of claim 11, having the sequence set forth in the sequence listing as SEQ ID NO:4.
14. A purified antibody which specifically binds to the polypeptide of claim 13.
15. An isolated nucleic acid comprising the nucleic acid set forth in the Sequence Listing as SEQ ID NO: 5.
16. The isolated nucleic acid of claim 15 in a vector suitable for expressing the nucleic acid.
17. The vector of claim 16 in a host suitable for expressing the nucleic acid.
18. A purified DRL polypeptide encoded by the nucleic acid of claim 15.
19. An isolated nucleic acid encoding the polypeptide of claim 18.
20. The polypeptide of claim 18, having the sequence set forth in the sequence listing as SEQ NO:6.

21. A purified antibody which specifically binds to the polypeptide of claim 20.
22. An isolated nucleic acid that encodes amino acid residues 65 to 250 of DRL as set forth in SEQ ID NO:2.
23. A polypeptide encoded by the nucleic acid of claim 22.
24. A purified antibody which specifically binds to the polypeptide of claim 23.
25. The isolated nucleic acid of claim 1 further comprising a tag.
26. The tag of claim 25, wherein the tag is a FLAG tag.
27. The tag of claim 25, wherein the tag is a human IgG Fc.
28. A polypeptide encoded by the nucleic acid of claim 25.
29. An isolated nucleic acid encoding the polypeptide of claim 28.
30. A purified antibody which specifically binds to the polypeptide of claim 28.
31. The isolated nucleic acid of claim 8 further comprising a tag.
32. The tag of claim 31, wherein the tag is a FLAG tag.

33. The tag of claim 31, wherein the tag is a human IgG Fc.
34. A polypeptide encoded by the nucleic acid of claim 31.
35. An isolated nucleic acid encoding the polypeptide of claim 34 .
36. A purified antibody which specifically binds to the polypeptide of claim 34.
37. The isolated nucleic acid of claim 15 further comprising a tag.
38. The tag of claim 37, wherein the tag is a FLAG tag.
39. The tag of claim 37, wherein the tag is a human IgG Fc.
40. A polypeptide encoded by the nucleic acid of claim 37.
41. An isolated nucleic acid encoding the polypeptide of claim 40 .
42. A purified antibody which specifically binds to the polypeptide of claim 40.
43. The isolated nucleic acid of claim 22 further comprising a tag.
44. The tag of claim 43, wherein the tag is a FLAG tag.
45. The tag of claim 43, wherein the tag is a human IgG Fc.

46. A polypeptide encoded by the nucleic acid of claim 43.
47. An isolated nucleic acid encoding the polypeptide of claim 46 .
48. A purified antibody which specifically binds to the polypeptide of claim 46.
49. An isolated double-stranded nucleic acid amplified using primer pair 5'-TATCGAATTCACAACCTTCTTCCCTTCTGCACCA(SEQ ID NO:7) and 5'-TATCGAATTCAATGAAAAGGGAAAAGTGAGGAACG (SEQ ID NO:8) consisting of 1) single-stranded DNA which has a molecular size of approximately 1.3 Kb and is derived from humans, and 2) a DNA complementary to the single-stranded DNA.
50. A single-stranded RNA corresponding to the single-stranded DNA of claim 49.
51. A single-stranded RNA corresponding to the single stranded complementary DNA of claim 49.
52. A polypeptide encoded by the isolated double-stranded nucleic acid of claim 49.
53. A method of stimulating an immune response in a subject by administering DRL-1 and a pharmaceutically acceptable carrier.
54. A method of suppressing an autoimmune response in a subject by administering an antibody to DRL-1 and a pharmaceutically acceptable carrier.

55. A method of inhibiting a T cell response by administering an antibody to DRL-1 and a pharmaceutically acceptable carrier.
56. The method of claim 55, wherein the T cell response is associated with transplantation rejection.
57. The method of claim 55, wherein the T cell response is associated with graft-versus-host responses.
58. A method of inducing apoptosis in a population of Tcells by administering DRL-1 to the cells.
59. A method of inhibiting apoptosis in a population of T cells by administering an antibody to DRL-1.
60. A method of stimulating the membrane bound form of DRL-1 by administering an antibody to DRL-1.
61. A method of screening for a DRL-1 receptor agonist comprising:
- a) contacting cells that express the DRL-1 receptor with a putative agonist.
 - b) detecting an increase in apoptosis in the cells of step (a), whereby the increase in apoptosis indicates a putative agonist having the potential to induce apoptosis; and

c) determining that the putative agonist identified in step (b) induces apoptosis by binding with DRL-1 receptor by competitive binding assays, thereby identifying a DRL-1 receptor agonist.

62. A method of screening for a DRL-1 receptor antagonist comprising:

a) contacting cells that express the DRL -1 receptor with a putative antagonist in the presence of DRL-1;

b) detecting a decrease in apoptosis in the cells of step (a) whereby the decrease in apoptosis indicates a putative antagonist having the potential to inhibit apoptosis and;

c) determining that the putative antagonist identified in step (b) inhibits apoptosis by binding with DRL-1 receptor by competitive binding assays, thereby identifying a DRL-1 receptor antagonist.

63. A method of stimulating an immune response in a subject by administering DRL-2 and a pharmaceutically acceptable carrier.

64. A method of suppressing an autoimmune response in a subject by administering an antibody to DRL-2 and a pharmaceutically acceptable carrier.

65. A method of inhibiting a T cell response by administering an antibody to DRL-2 and a pharmaceutically acceptable carrier.

66. The method of claim 65, wherein the T cell response is associated with transplantation rejection.

67. The method of claim 65, wherein the T cell response is associated with graft-versus-host responses.
68. A method of inducing apoptosis in a population of T cells by administering DRL-2 to the cells.
69. A method of inhibiting apoptosis in a population of T cells by administering an antibody to DRL-2.
70. A method of stimulating the membrane bound form of DRL-2 by administering an antibody to DRL-2.
71. A method of screening for a DRL-2 receptor agonist comprising:
- a) contacting cells that express the DRL-2 receptor with a putative agonist.
 - b) detecting an increase in apoptosis in the cells of step (a), whereby the increase in apoptosis indicates a putative agonist having the potential to induce apoptosis; and
 - c) determining that the putative agonist identified in step (b) induces apoptosis by binding with DRL-2 receptor by competitive binding assays, thereby identifying a DRL-2 receptor agonist.
72. A method of screening for a DRL-2 receptor antagonist comprising:
- a) contacting cells that express the DRL-2 receptor with a putative antagonist in the presence of DRL-2;

b) detecting a decrease in apoptosis in the cells of step (a) whereby the decrease in apoptosis indicates a putative antagonist having the potential to inhibit apoptosis and;

c) determining that the putative antagonist identified in step (b) inhibits apoptosis by binding with DRL-2 receptor by competitive binding assays, thereby identifying a DRL-2 receptor antagonist.

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1 MPASSPFL LAPKGPPGNMGGPVREPALSVALWLSWGAALGAVACAMALLTQQTELOSLRREVSRLQG
 68 TGGPSQNGEGYPWQSLPEQSSDALEAWESGERSRKRRAVL TQKQKKQHSVLHLVPINATSKDDSDVT
 135 EVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDVTFTMGQVVSREGQGRQETLFR CIRSM
 201 PSHPDRAYNCSYAGVFHLHQGDILSVIIPRARA KLNLSPHGTF LGFVKL

FIG. 1A

120	LVPI-NATSKDDSDVTEVMWQPALRRGRGLQAQGYGVR-----IQDA	DRL
143	LKVAHLTGKSNRSRSMLEWED--TYGIVLL-SGVKYKKG-GLVINET	Fas ligand
87	-KPVAVHVANPQAEQ-QLQWLN--RRANALLANGVELRDN-QLVVPSE	TNF- α
61	LKPAHLIGDPSKQN-SLLWRA--NTDRAFLQDGFSLSN-SLLVPTS	Lymphotoxin
87	L-PAHLIGAPLK-GQGLGNET--TKEQAFLTSGTQFSDAEGALPQD	Lymphotoxin β

161	GVYLLYSQVLFQD-----VTFMGQVVS RFGQGRQETL	DRL
187	GLYFVYSKYVFRGQSC--NN-----LPLSHKVMRNSKYPQDLV--M	Fas ligand
130	GLYLLYSQVLFKGGGC-----PSTHMLTHTLSR AVSYQTKVN--L	TNF- α
105	GLYFVYSQVVFSGKAY--SPKATSSPLYLAHEVQLFSSQYPFHVP--L	Lymphotoxin
131	GLYLYCLVGYRGRAPPGGGDPQGRSVTLRSSLYRAGGAYGPGTPELL	Lymphotoxin β

194	FRCLRSM--PSHPDRA-----YNCSYAGVFHLHQGDILSVIIPRA	DRL
225	MEGKMMSYC-----TTGQMWARSSYLGA VFNLTSADHLYVNVSEL	Fas ligand
170	LSALKSPCQRETPE--GAEAKPWYEPIYLGGVFQLEKGDRLSAELNRP	TNF- α
149	LSSQKMVYP-----GLQEPWLHSMYHGA AFQLTQGDQLSTHTDGL	Lymphotoxin
179	LEGAETVTPVLDPARQGGYGPLWYTSVGFGGLVQLRRGERVYVNISHP	Lymphotoxin β

233	RAKLNLSPHG-TFLGFVKL.	DRL
265	-SLVNFEES-QTFFGLYKL.	Fas ligand
216	-DYL DFAESGQVYFGI IAL.	TNF- α
189	-PHVLSPS-TVFFGAFAL.	Lymphotoxin
227	-DMVDFAR-GKTFFGAVMVG.	Lymphotoxin β

FIG. 1B

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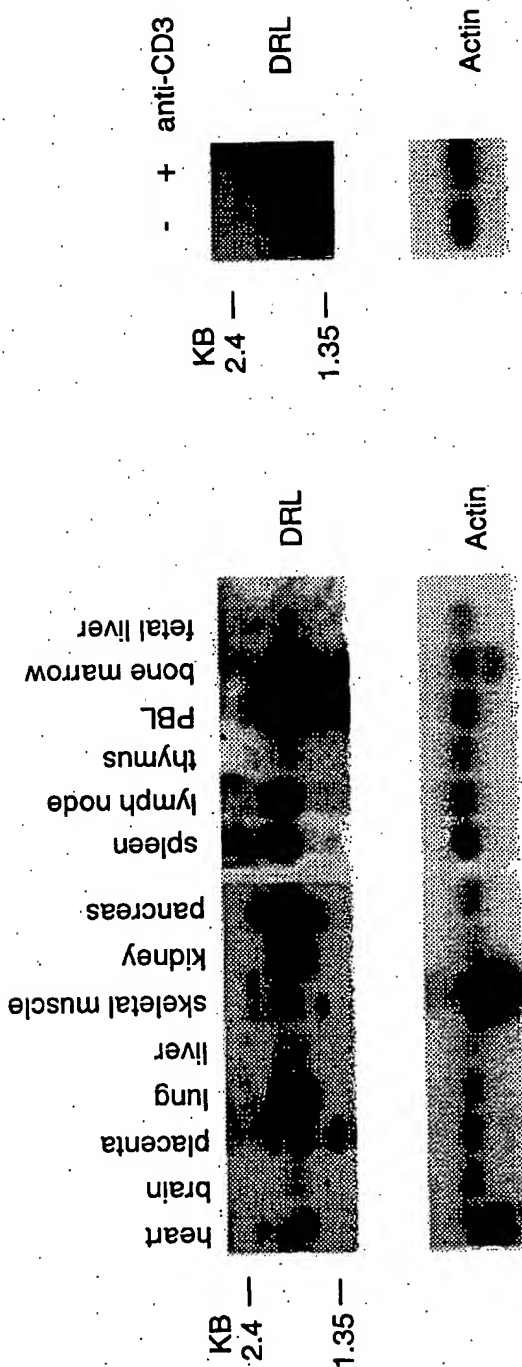


FIG. 2B

FIG. 2A

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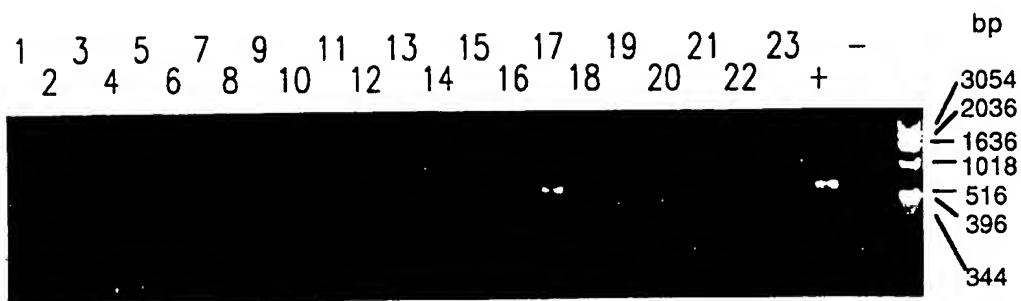


FIG.3A

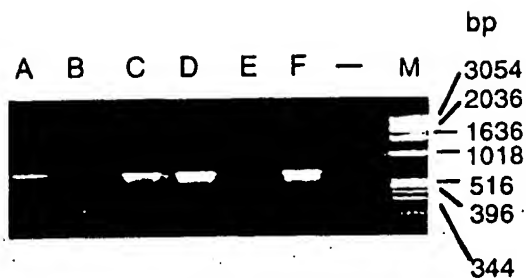


FIG.3C

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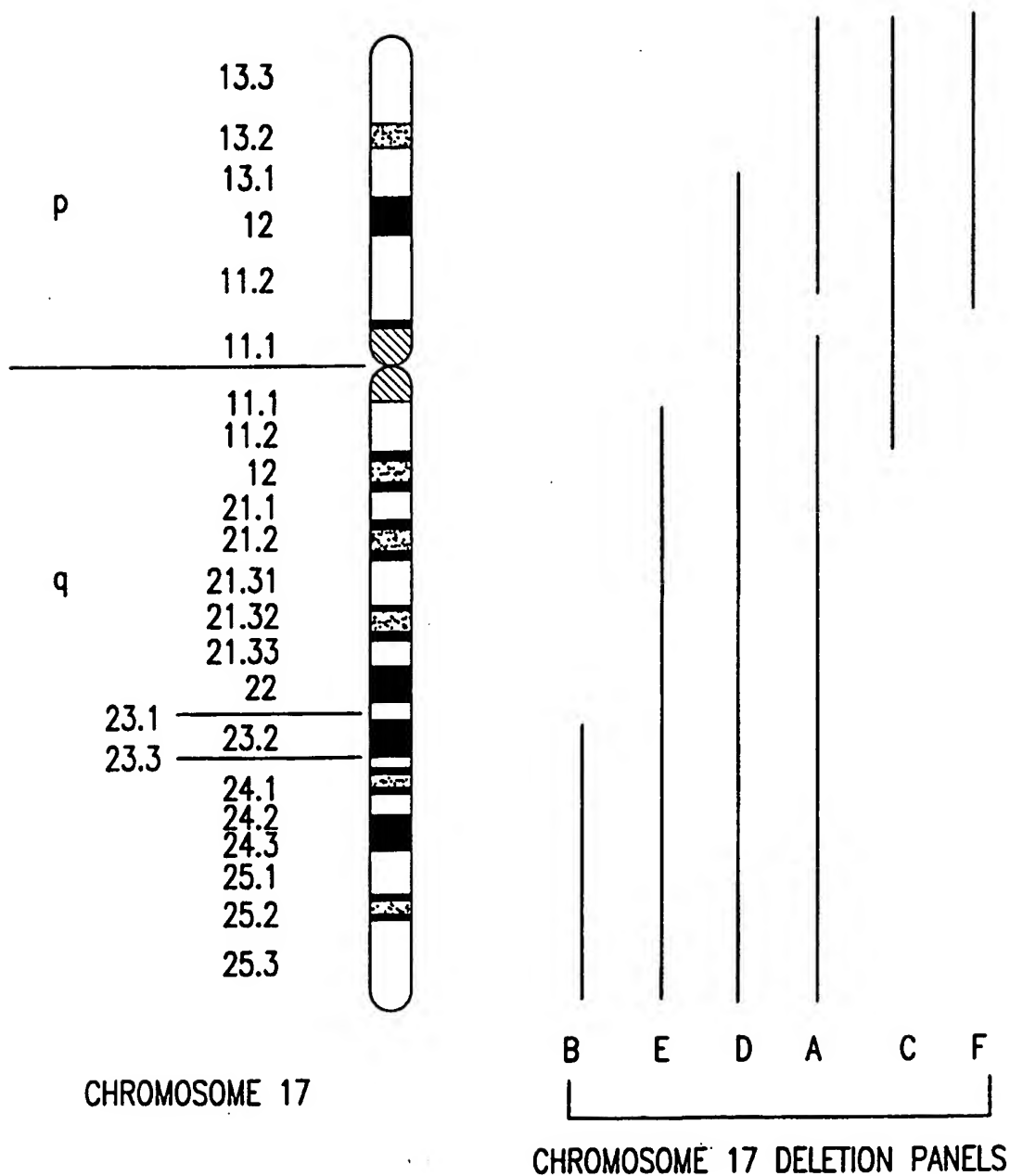


FIG.3B

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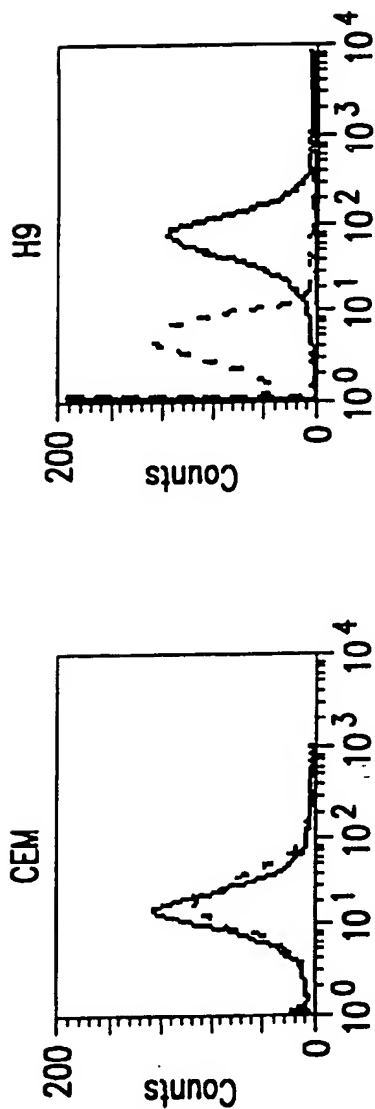


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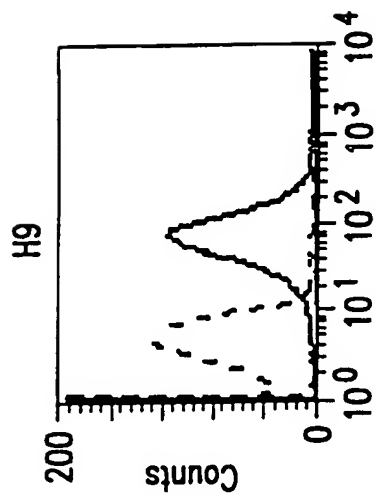


FIG. 4C

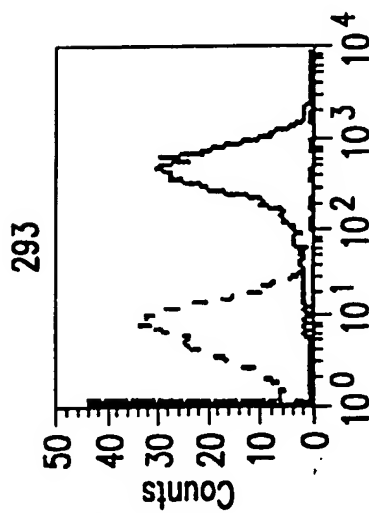


FIG. 4B

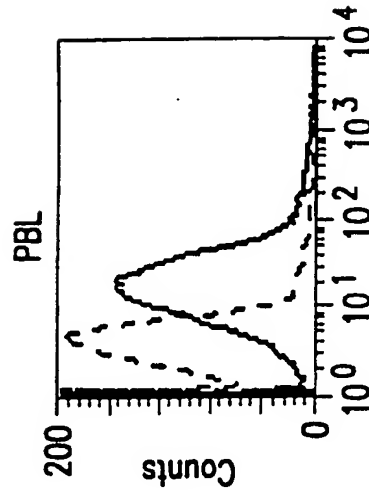


FIG. 4D

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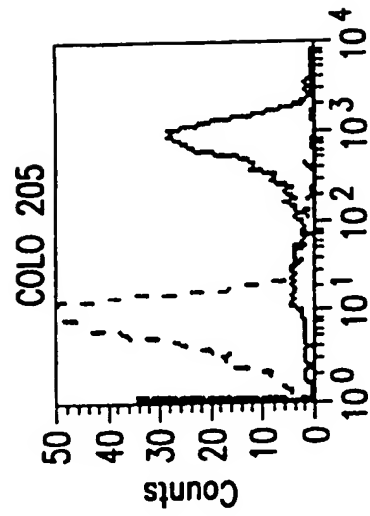


FIG.4G

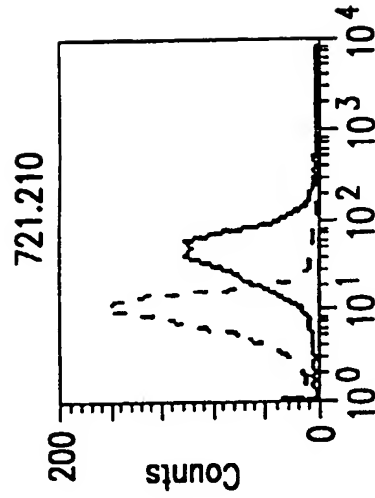


FIG.4H

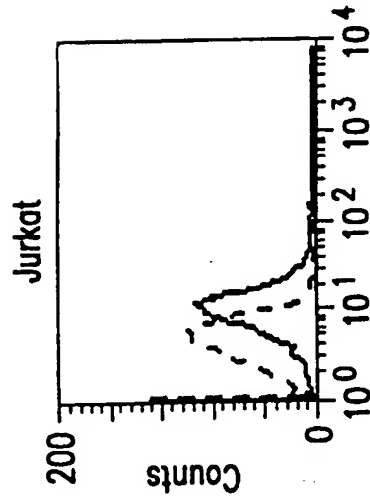


FIG.4E

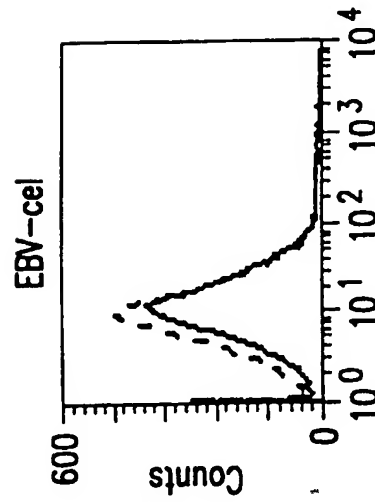


FIG.4F

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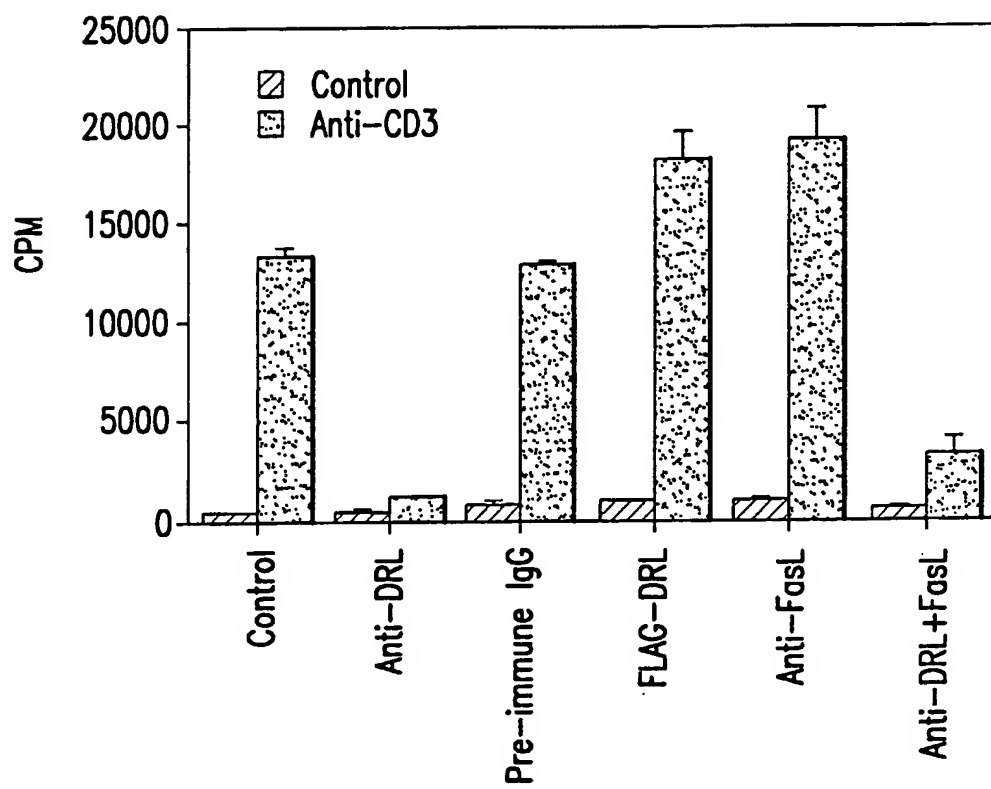


FIG.5

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Q L I A D S E T P T I Q K G S Y T F V P W L L S F K F G S A L E E K E N K I L V K E
T G Y F F I Y G Q V L Y T D K T Y A M G H L I Q F K K V H V F G D E L S L V T L F
P C I Q N M P E T L P N N S C Y S A G I A K L E E G D E L Q L A I P F E N A Q I S L
D G D V T F F G A L K L L.

FIG.6

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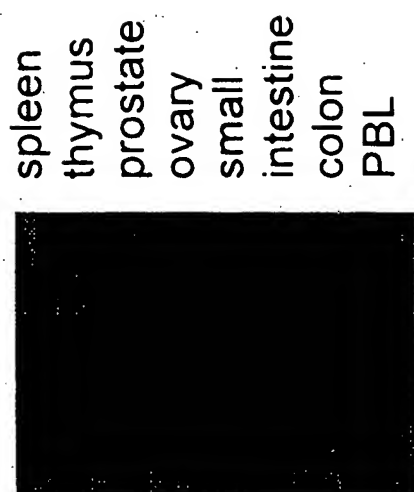
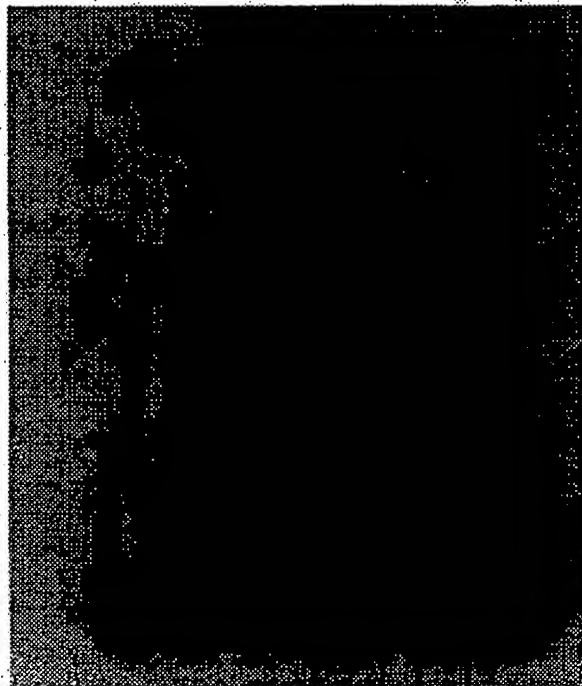


FIG.7

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1. 2. 3. 4. 5. 6. 7.



1. PCDNA3 only
2. DRL-1
3. DRL-2 clone 1
4. DRL-2 clone 2
5. DRL-2 clone 3
6. DRL-2 clone 4
7. No DNA

FIG.8

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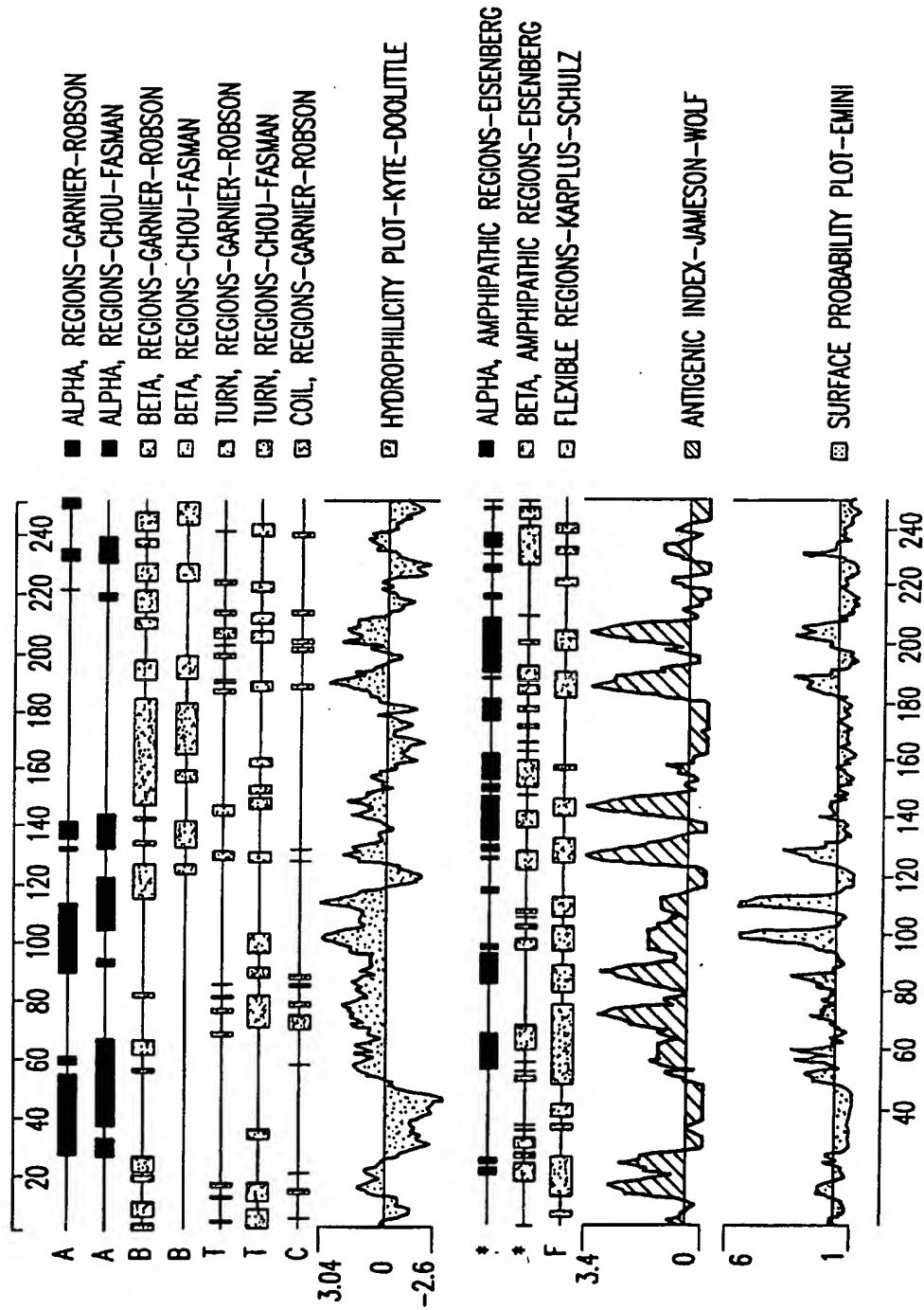


FIG.9

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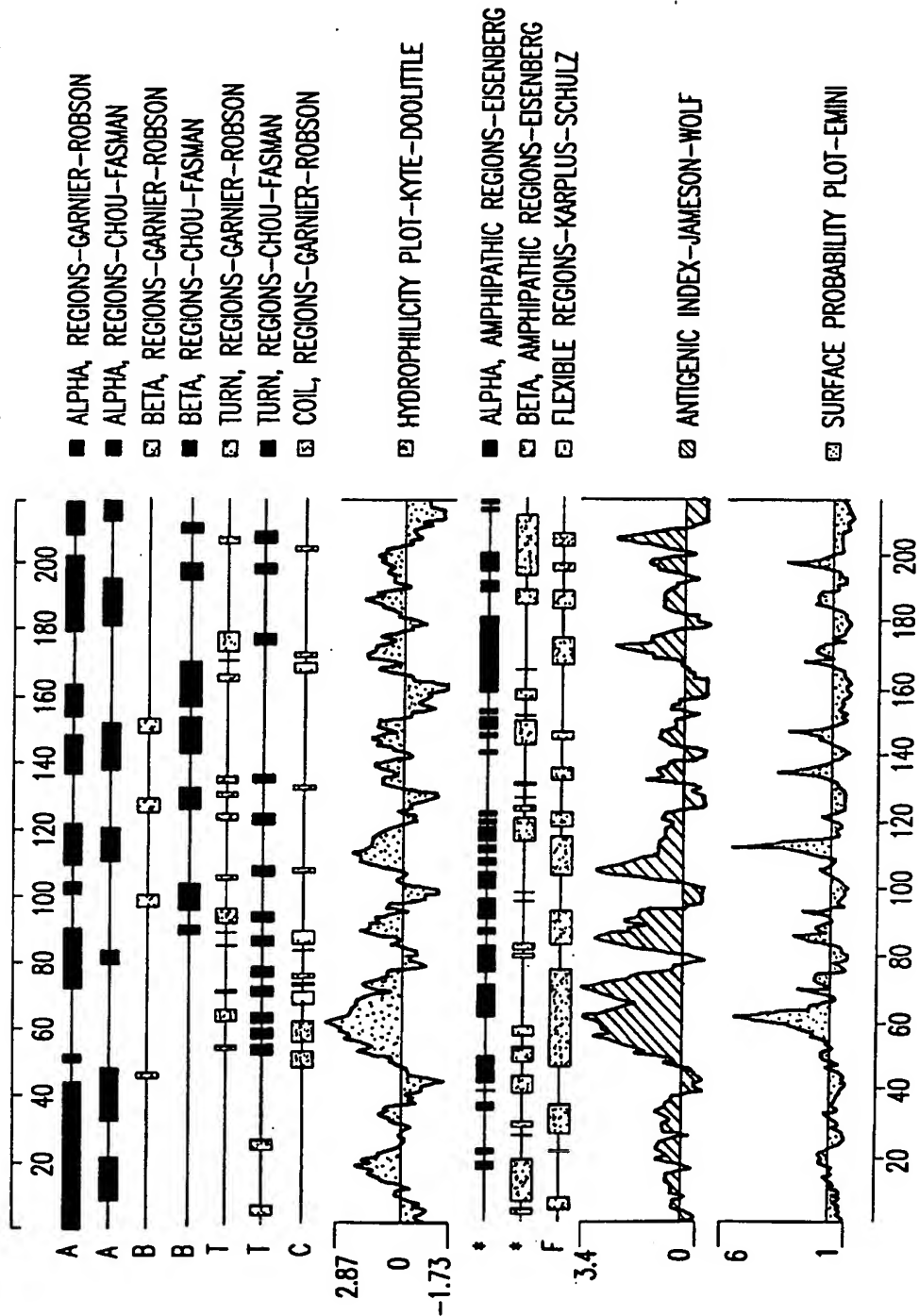


FIG.10

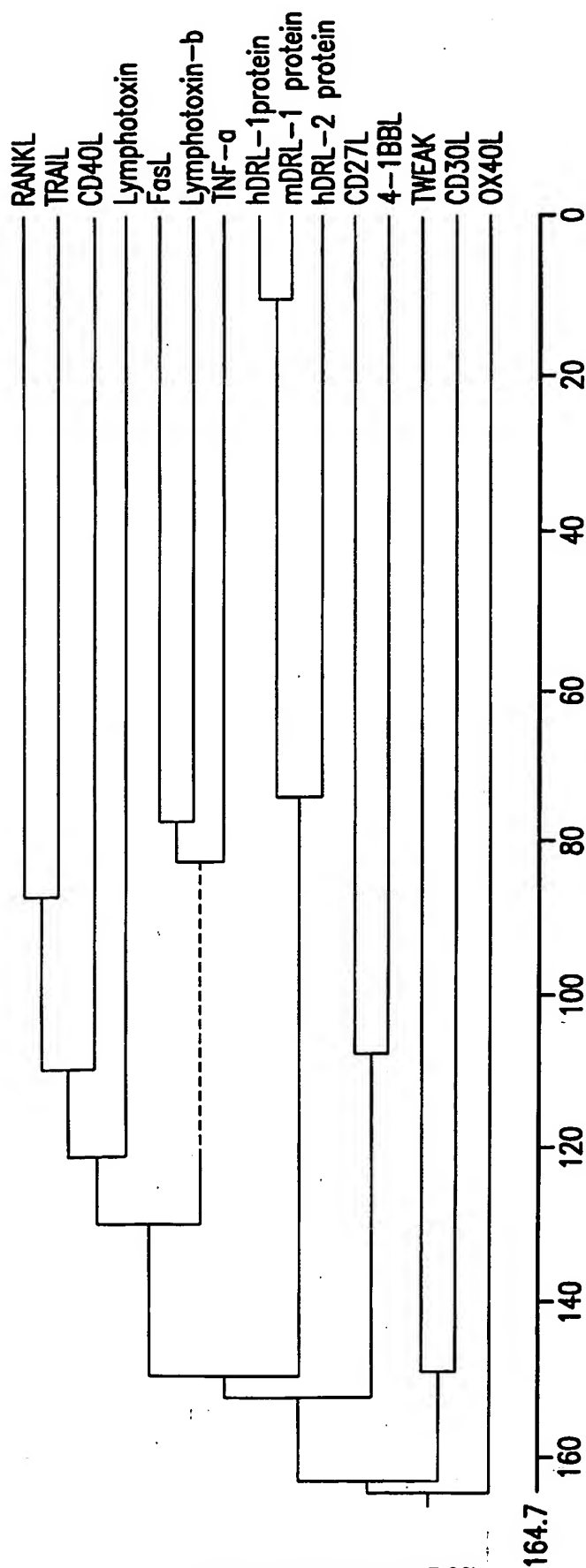


FIG.11

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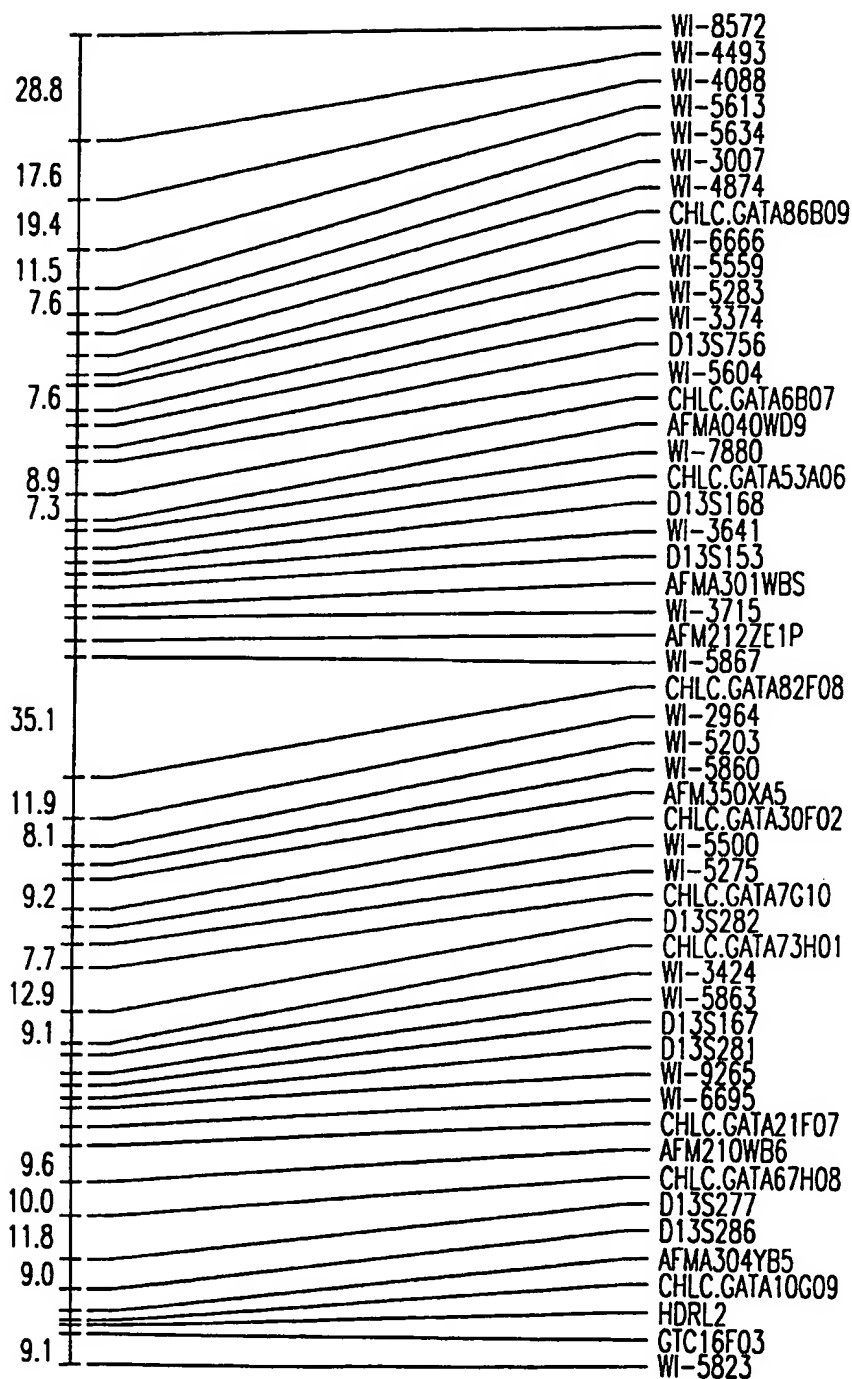


FIG.12

SEQUENCE LISTING

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Lenardo, Michael J.
Wang, Jin
Jiang, Di

<120> A NOVEL TUMOR NECROSIS FACTOR FAMILY MEMBER DRL AND RELATED COMPOSITIONS AND METHODS

<130> 14014.0354/P

<150> 60/106,976

<151> 1998-11-04

<160> 10

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<211> 753

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			20					25				30			
Leu	Ser	Trp	Gly	Ala	Ala	Leu	Gly	Ala	Val	Ala	Cys	Ala	Met	Ala	Leu
		35					40					45			

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 85 90 95
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 Lys Gln His Ser Val Leu His Leu Val Pro Ile Asn Ala Thr Ser Lys
 115 120 125
 Asp Asp Ser Asp Val Thr Glu Val Met Trp Gln Pro Ala Leu Arg Arg
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 Gly Val Tyr Leu Leu Tyr Ser Gln Val Leu Phe Gln Asp Val Thr Phe
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 Thr Met Gly Gln Val Val Ser Arg Glu Gly Gln Gly Arg Gln Glu Thr
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 195 200 205
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 Leu Ile Ala Asp Ser Glu Thr Pro Thr Ile Gln Lys Gly Ser Tyr Thr
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 180 185 190
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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/25954

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07K14/525

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

STRAND, EPO-Internal, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HAHNE M ET AL: "APRIL, a new ligand of the tumor necrosis factor family, stimulates tumor cell growth" JOURNAL OF EXPERIMENTAL MEDICINE, JP, TOKYO, vol. 188, no. 6, 21 September 1998 (1998-09-21), pages 1185-1190-1190, XP002099001 ISSN: 0022-1007	1-7, 15-26, 28-30, 37,38, 40-44, 46-53
Y	Cited mainly against inventions 1 and 3 the whole document --- -/--	25-30, 32, 37-48, 54-62

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

13 July 2000

Date of mailing of the international search report

21 07. 00

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 99/25954

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 33902 A (HUMAN GENOME SCIENCES INC ;NI JIAN (US); GENTZ REINER L (US); YU G) 18 September 1997 (1997-09-18)	1-7, 15-24, 49-62
Y	Cited mainly against inventions 1 and 3 page 4, line 1 -page 8, line 5; claims 1-30; figure 1	25,26, 28-30, 37,38, 40-44, 46-48, 54-62
Y	WO 94 19463 A (AUCKLAND UNISERVICES LTD ;CROSIER PHILIP STANLEY (NZ); CROSIER KAT) 1 September 1994 (1994-09-01) Cited against all inventions page 34, line 1 - line 13	27,33, 39,45
P,X	EP 0 919 620 A (LILLY CO ELI) 2 June 1999 (1999-06-02) Cited against inventions 1 and 3 the whole document	1-7, 15-30, 37-62
P,X	WO 99 11791 A (UNIV WASHINGTON) 11 March 1999 (1999-03-11) Cited against inventions 1 and 3 the whole document	1-7, 15-30, 37-62
P,X	WO 99 28462 A (BAKER KEVIN P ;CHEN JIAN (US); GENENTECH INC (US); YUAN JEAN (US);) 10 June 1999 (1999-06-10) Cited against inventions 1 and 3 the whole document	1-7, 15-30, 37-62
P,X	WO 98 55620 A (MASIAKOWSKI PIOTR ;REGENERON PHARMA (US); VALENZUELA DAVID (US)) 10 December 1998 (1998-12-10)	8-14,31, 33-36, 63-72
P,Y	Cited against invention 2 the whole document	32
P,X	WO 98 55621 A (MASIAKOWSKI PIOTR ;REGENERON PHARMA (US); VALENZUELA DAVID (US)) 10 December 1998 (1998-12-10)	8-14,31, 33-36, 63-72
P,X	Cited against invention 2 the whole document	32
X	EP 0 869 180 A (SMITHKLINE BEECHAM CORP) 7 October 1998 (1998-10-07)	8-14,31, 34-36, 63-72
Y	Cited against invention 2 page 13, line 39 page 14, line 10 - line 46 page 18; claims 1-19	32,33

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/25954

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 53-62 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-7,22-30,43-52 (completely); 53-62 (partially)

A nucleic acid encoding a TNF-related polypeptide, human DRL-1 (SEQ ID NO:1) and subject-matter related thereto.

2. Claims: 8-14,31-36,63-72 (completely)

A nucleic acid encoding a TNF-related polypeptide, human DRL-2 (SEQ ID NO:3) and subject-matter related thereto.

3. Claims: 15-21,37-42 (completely); 53-62 (partially)

A nucleic acid encoding a TNF-related polypeptide, mouse DRL-1 (SEQ ID NO:5) and subject-matter related thereto.

INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No

PCT/US 99/25954

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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